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REVIEW ON THE DEVELOPMENT OF ANTIBIOTICS INDUSTRY IN SELECTED COUNTRIES

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Technical report: Antibiotic industry in Japan, history and development*

Prepared by the United Nations Industrial Development Organization

Based on the work of Minoru Aizawa, UNIDO consultant

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PREFACE

This case study on antibiotics industry in Japan, history and development is part of an effort by the UNIDO Secretariat to undertake case studies which would review the development of this important industry in selected developed and developing countries with the aim of promoting the growth and development of antibiotics industry in developing countries. The present study highlights the outstanding progress achieved in Japan in terms of research and development towards new antibiotics.

The discovery of a broad variety of therapeutic agents to treat a number of pathologic conditions and the launching of medicaments utilized to treat or cure a large number of ailments had a strong impact on the world consumption of pharmaceuticals. The structure of world consumption of pharmaceuticals presents an interesting pattern in a large number of countries. The incidence of a broad variety of microbial diseases places the antimicrobials, and out of this group, the antibiotics as the leader in world consumption. While systemic antibiotics ranked during 1979 No. 1 in Brazil, Japan, Pakistan, Philippines and Venezuela, they ranked No. 3 in the U.S.A. and No. 7 in the Federal Republic of Germany. The market share of systemic antibiotics during the same year in developing countries was 25 percent in Pakistan, 19 percent in Philippines, and 14 percent in Brazil and Venezuela. As regards developed countries, the market share of systematic antibiotics in 1979 was 26 percent in Japan, 7 percent in the U.S.A. and 4 percent in the Federal Republic of Germany. While the value of world wide consumption of antibiotics in 1980 was $2,259 millions, the share of developing countries was only 20.05 percent.

With the exception of a few, most of the developing countries totally depend on the import of antibiotics either in the form of finished products or as active ingredients to produce pharmaceuticals in dosage form. The non-availability of adequate amounts of convertible currency is limiting the imports of these essential drugs, which in turn adversely affect the health care programmes in these countries . Further several raw materials used in the manufacture of antibiotics are products of agriculture and these are available in plenty in most of the developing countries. In

view of this local production of antibiotics could result in achieving self reliance in this area to the extent feasible.

Some developing countries in Latin America, Asia and few in Africa have some base and infrastructure necessary for the production of antibiotics through fermentation and these include Algeria, Argentina, China, Cuba, Egypt, India, Iraq, Mexico, Pakistan, Peru, Republic of Korea and Thailand. While this industry is at initial stages of development in some of these countries, it has made significant progress in others. It is also proposed to carry out the case study on antibiotics industry in one of the latter developing countries.

The study was prepared by Minoru Aizawa, Adviser for Overseas Operation and ex-Managing Director of Toyo Jozo Co. Ltd., Japan, which made a significant contribution to the development of this industry world wide.

SUMMARY

The antibiotics industry in Japan virtually came into existence with the production of Penicillin just after World War II with the assistance of the allied occupation forces. Subsequently production of Streptomycin, Chlorampenicol and Tetracycline was taken up under licensing agreements. Later on new antibiotics of Japanese origin such as Colistin, Kitasamycin, Kanamycin followed by Mitomycin, Bleomycin the anticancer antibiotics came on the scene. This trend led to the production of many new Beta-Lactam compounds based on 6-APA and 7-ACA.

Major Japanese antibiotic producers may be broadly classified into three categories - a) authentic local pharmaceutical industries, b) foreign subsidiaries/joint ventures and c) local industries other than pharmaceutical. In total, 73 companies were listed in 1982. The gross output of antibiotic drugs in 1982 was 865,148 million Japanese Yen (US$ 3,366 million) which amounted to 21.7 percent of the total output of pharmaceutical products in Japan - 3,980,232 million Japanese Yen (US$ 15,487 million).

Beta-lactam family and the aminoglucoside family constitute major groups of antibiotics currently used in Japan. B-lactam antibiotics are the derivatives of a bicyclic ring system. The Penicillin ring system called Penam contains a four membered beta-lactam ring fused with a five membered thiazolidine ring, whereas the cephalosporin ring system, 3 cephem contains the same beta-lactam ring fused with an unsaturated six-membered dihydrothiazine ring. According to the structure-activity relationships, the Penam family members can be characterized in four sub-groups - a) oral form, b) anti-staphylococcal - beta-lactamase compounds, c) wide spectrum compounds and d) anti-pseudomonas compounds. The cephem compounds have been divided into three categories according to the generation in response to the selective clinical use - the first generation group is susceptible to hydrolysis by cephalosporinase, while the second generation is free from such susceptibility and the third generation is of anti-pseudomonal activity as well as resistant to the cephalosparinise.

The Aminoglycoside family may be divided into two sub groups - a) presence of furanose unit and b) absence of furanose unit.

Novel compounds are derived from the parent antibiotic in terms of the chemical modification leading to a more potent structure. The method of modification may be based on a chemical reaction or microbial conversion or a combination of both. The novel antibiotics derived from chemical modification are termed semi-synthetic compounds.

The discovery of a new antibiotic primarily depend on the microorganisms producing the antibiotic compound."The method of screening for such specific microorganisms,therefore, has been the key technology necessary for the finding of a new antibiotic. Special screening methods for specific antibiotics are discussed.

CHAPTER I: HISTORICAL REVIEW ON THE DEVELOPMENT OF ANTIBIOTIC INDUSTRIES IN JAPAN

Table 1 presents a full member of the antibiotic producers who have been licensed from Japanese Government as official supplier for the Social Security Organization. By these companies, a variety of antibiotic drugs are manufactured in conformity with G.M.P. legislated by the Government in the form of injectables and oral drugs ; In general, drugs for topical use are excluded from this channel.

Major antibiotic producers may be divided into three representative groups in association with the historical development of the antibiotics in this country; a) Authentic local pharmaceutical industries, such as Banyu, Chugai, Daiichi, Fujisawa, Sankyo, Shionogi, Takeda and Yamanouchi, b) Foreign pharmaceutical industries, in the name of Japan Limited, for example, Beecham, Bristol, Ciba-Geigy, Essex, Glaxo, Hoechst, Lederle, Pfizer, Roussell,Squibb, Upjohn and Wyeth, and c) Local industries other than pharmaceutical, for instance, Kanebo(Textile), Kyowa Hakko(Alcohol), Meiji Seika(Confectionery), Nihon Kayaku(Explosives), Sanraku-Ocean(Alcohol), Sumitomo Kagaku(Heavy Chemicals) and Toyo Jozo(Alcohol).

In total, 71 companies were listed in 1982.

The gross output of antibiotic drugs was 865,148 MJY(Million Japanese Yen) in 1982, sharing 21.7% of the total output of pharmaceutical products, 3,980,232 MJY. This was the top share among various types of drugs. The same trend had been kept in 1981(21.2%), and in 1980(23.4%), respectively.

The licensing office announced the capacity of the medicational experts as follows in 1982: 167,952 physicians, 58,362 dentists and 124,390 pharmacists. The antibiotic industries are now at their best in Japan.

However, from point of view of historical background, it should be noted that this successful industry was just founded after World War II by the policy of G.H.Q. of allied occupied forces, along with their own necessity for the local supply of the penicillin drugs. The new project thus suggested seemed a very interesting one for then jobless industries, therefore, more than 70 companies made submission for Government approval in order to take part in the new project. However, the project called for

the hitherto inexperienced wide-range scientific integrity for its performance based on the biological, chemical, mechanical, electromechanical, and pharmaceutical requirements, altogether.

Strange to say, most of the drug companies did not take interest in joining the project, except a few of them, perhaps because they did not possess clear idea about fermentation. On the other hand, companies without pharmaceutical background, such as Meiji-Seika(Confectionery), Meiji-Nyugyo(Milk Industry), Nihon Kayaku(Explosives and Dyestuff), Kaken Kagaku (Division of Riken Research Institute) and Toyo Jozo(Alcohol Industry) pushed on the project strongly, taking advantage of the fermentation know-how hitherto accumulated.

Consequently, 32 different brands appeared in the market initially, selling crude penicillin injectables, but, because of the price reduction due to severe competition, in ten years, many of the participants have discontinued the project, some switched over to a new antibiotic, and some made withdrawal from the pharmaceutical industry. After then, consistent competitiveness severely spread over the global level has eliminated the penicillin producer one after another, and finally, Meiji Seika and Toyo Jozo are still in a position of surviver.

It is worthy to note that the foundation of "Penicillin Research Association"(Nihon Penicillin Gakujutsu Kyogikai) which has been renamed "Antibiotic Research Association, Japan" later on, was made soon after the introduction of the penicillin project. The association was sponsored by the project participants and headed by the late Mr. Yukimasa Yagisawa, son of the leading medical professor of Tokyo University, and a fluent English speaker. He exhibited an outstanding ability in managing good coordination among Government officers, medical professors, research leaders and plant technologists, in order to make rapid access to the performance as a whole. For this purpose, he organized several sessions, such as technical, mechanical and clinical, under the participationof the experts from each corresponding field. This was useful to propagate the guidance of the project. Discussions in such sessions were published and distributed in the form of "Journal of Penicillin", the origin of "Journal of Antibiotics", now world-widely accepted as international standard.

After the penicillin project, Streptomycin(Meiji Seika, Kyowa Hakko, Kaken Kagaku and Nikken Kagaku), Chloramphenicol(Sankyo) and Tetracyclines (Takeda and Taito, later on taken over by Pfizer) came in the antibiotic market, among which chloramphenicol was unique because of entirely chemical synthesis without any process of fermentation.(cf; Table 2)

These new antibiotics were introduced on the basis of license agreement exclusively granted for the specified partners, therefore, the technology covering the manufacturing processes was kept in secrecy. Finally, Erythromycin(Shionogi) arrived at Japanese market.

However, initially monopolized broad spectrum antibiotics could not enjoy their privilege for a long time, because of complex situations of the patentability, for instance, Fujisawa got the license of marketing chloramphenicol from Karlo Elba in 1951, and Yamanouchi did the same from Boeringer in 1954, displacing Sankyo-Parke Davis as the sole distributor of this antibiotic. Meiji Seika also came in Chlortetracycline market in a different way in 1953, since they claimed an independent manufacturing process based on a novel variant of the antibiotic-producing strain.

Same is true with the case of erythromycin, Dainippon Seiyaku followed Shionogi in a yearly interval. Many antibiotic producers have learnt the necessity of their own new antibiotics under such confusing environments.

On the other hand, a variety of novel antibiotics of Japanese origin have been discovered by the consistent efforts of research activities which had been cultivated since the project of penicillin. Some major group of such antibiotics were introduced to the medical market, for instance, Colistin against gram-negative pathogens(Found by Dr. Y. Koyama in 1950, and identified as Polymixin E in 1963), this is the product of bacteria; Kitasamycin or Leucomycin, a 16-membered macrolide(Dr. T. Hata, 1953); Sarkomycin, the first oncostatic agent produced by *Streptomyces erythrochromogenes* obtained through screening against the *Yoshida sarcoma* using experimental animals(Dr. H. Umezawa, 1953); Mitomycin, also the oncostatic agent(Dr. T. Hata, 1956); Kanamycin, an aminoglycosidical antibiotic, effective against resistant strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis*(Dr. H. Umezawa, 1957) and Bleomycin, a novel oncostatic agent(Dr. H. Umezawa, 1962), and so on.

In 1955, clinical doctors were shaken up with the terrible cases caused by the anaphylactogenesis after the injection of penicillin.

However, quick response had been taken in the selection of the type of antibiotic as well as the form of administration in order to avoid such lethal side effect: Phenoxymethyl PC or PC-V was marketed in 1956 by several suppliers, since this type of PC enabled the oral administration because of acid-stability, and was believed less hazardous because PC-proteins complex might be destroyed by the gastric enzymes. At the same time, macrolide antibiotics did not miss the chance for taking over PC, Carbomycin(Pfizer) and Kitasamycin(Toyo Jozo) took part in this market together with Erythromycin of priority. Oleandomycin and Novobiocin did the same in 1957. Kanamycin appeared in 1958, the second major aminoglycosidic antibiotic, also enjoyed the chance to cover the therapeutic area of PC, needless to say, it was welcomed in treating tuberculosis caused by SM-resistant strains of Mycobacterium, as well.

Particular attention should be paid to the development of the oncostatic antibiotics(may be incorrect terminology, but generally accepted) carried out in Japan. Sarkomycin, though out of use nowadays, is the No. 1 substance discovered by the screening for this purpose. Mitomycin and Chromomycin-A3(Takeda, 1961) succeeded the position, until Bleomycin appeared in 1968. Then, Daunorubicin(1970) and Doxorubicin(1974) were introduced as foreign visitors from Farm Italia, pioneer of the anthracycline series of the oncostatic family. Neocartinostatin(1976) again resumed the national power in this field, followed by Peplomycin(1980), a variant of Bleomycin, and Aclarubicin(1981), a new derivative of the anthracycline series, now discovered in Japan.

With regard to the anti-tuberculosis antibiotics, Rifampicin(Repeti, 1971), and Enviomycin(Toyo Jozo, 1975) were added to the classic antibiotics, such as SM, KM, Cycloserine and Capreomycin. No more novel antibiotic is required in Japan, since the remarkable decrease has been characterized in the patients.

The advent of the semisynthetic PC was first recorded in 1959 in the name of Phenethicillin(Banyu, Taito-Pfizer), and Meiji Seika as well took part in the same market in 1960 under the license of Beecham. In accordance with the quick development of new PCs made by Bristol and Beecham, Methicillin(Banyu, Meiji Seika) was added in 1961, followed by Oxacillin

(Banyu, 1962), Propicillin(Meiji Seika, 1963), Ampicillin(Meiji Seika, Banyu, 1963), and Cloxacillin(Meiji Seika, Banyu, 1964).

On the other side, in 1965, Cephaloridine(Glaxo-Torii) and Cephalothin(Eli-Lilly-Shionogi) opened the cephalosporin market in Japan. Cephaloglycin followed in 1969, and Cephalexin in 1970, then Cefazolin(Fujisawa), the first cephem compound created by the national research activity. This unique substance exhibited almost complete stability to staphylococcal lactamases and attained world-wide acceptance in challenging the resistant strains of *Staphylococcus aureus*. This event has encouraged a whole national research activity toward further discovery of new beta-lactam compounds calling forth fruitful results, such as, Sulbenicillin(Takeda, 1972), Talampicillin(Yamanouchi, 1977), Ceftezole(Fujisawa, 1977), Piperacillin(Toyama Kagaku, 1979), Cefmetazole(Sankyo, 1979), Cefsulodin, Cefotiam(Takeda, 1980), Latamoxef(Shionogi, 1981), Ceftizoxime(Fujisawa, 1981), Cefoperazone (Toyama Kagaku, 1981), Cefmenoxime(Takeda, 1982) and Cefotetan(Yamanouchi, 1983). On the other hand, it is also important to set focus on Kanamycin derivatives, since the chemical modification of KM has been carried out on the basis of scientific approach to the mechanisms of bacterial resistancy against KM in terms of molar biology.(See, Chapter III for details) The research group headed by Dr. H. Umezawa revealed how KM was biologically inactivated by such bacteria, and they modified KM molecule so as to be free of such inactivations. These findings reflect on a series of KM derivatives represented by Dibekacin(Meiji Seika, 1974). Amikacin(Bristol-Banyu, 1976) was also developed from a different line of scientific approach of such mechanisms of resistancy. Meanwhile, Gentamicin was introduced in 1968, taking advantage of the sensitivity against KM-resistant pathogens as well as *Pseudomonas aeruginosa*.

Another topic of significance in research intensity may be the history of macrolide antibiotics, as mentioned before, the basic macrolides were introduced in considerably early stage(EM, OLM and KTM), then Spiramycin (Rhone- Pulenc-Kyowa Hakko) was added to this market in 1963, followed by Josamycin(Yamanouchi) in 1969, which was found to be identical with KTM A3 in 1970. Complex interrelationships among 16-membered macrolide compounds

have drawn attention of various research institutes working for the new antibiotics, and ended up with the discovery of several new compounds of this family closely related to KTM molecule in 1970s. Midecamycin(Meiji Seika, 1973) and Maridomycin(Takeda, 1975) were thus added to the market.

Finally, the current trend of major antibiotics in Japan may be estimated by the size of production of injectable form among aminoglycosides(AG), penam- and cephem-seriesas follows:

AG : Penam : Cephem = 1 : 6.5 : 20.6

CHAPTER II: INTRODUCTION OF VARIOUS ANTIBIOTICS CURRENTLY MARKETED IN JAPAN

In the previous chapter, the scope of antibiotic drugs has been outlined under the focus of historical background. It may be concluded that the modern chemotherapy is largely enjoying the progress in particular type of antibiotic groups, because of the versatile response for therapeutical demands. Typical representatives of such group will be the beta-lactam antibiotic family membered with cephem- and penam-series, since most of the pathogenic organisms including antibiotic-resistant strains, are sensitive to this type of antibiotics.

In Table 3, a variety of the antibiotic drugs are listed according to the information published by Japanese Government for Social Security in 1983. There are two major divisions according to the form of administration, such as injectables and oral drugs, with the subdivision in terms of the antibiotic spectrum. A topical remedy is excluded from the category of Social Security except particularly specified cases.

It is clearly seen from the Table that the cephem compounds(22 individuals) and the penam compounds(also 22 individuals) keep the top share(classic penicillins are excluded), then follow the oncostatic agents(9), most of which have been discovered and developed in Japan, and the aminoglycosides(8) come next. Tetracyclines(7) and macrolides(6) are used as wide-spectrum antibiotics, and 6 different anti-Mycobacteriums are exclusively used against tuberculosis, and 3 antibiotics remain for the antifungal remedy.

In this chapter, in accordance with the keen demands from modern chemotherapy, detailed review will be made along with the line of major antibiotic groups, such as the beta-lactam family as well as the aminoglycoside family.

Section 1. Beta-lactam Antibiotics

Chemically, this type of antibiotics is the derivative of a bicyclic ring system: The penicillin(PC) ring system called penam, contains a four-membered beta-lactam ring fused with a five membered thiazolidine ring, whereas the cephalosporin(CPN) ring system, 3-cephem, differs insofar

as the same beta-lactam ring is fused with an unsaturated six-membered dihydrothiazine ring.(cf: Fig. 1) The beta-lactam ring is possessed chemically in common with these compounds, and the classification was made according to unusual characteristic of the molecules[1].

The beta-lactam antibiotics(referring only to penams and cephems here) are synthesized by only a few microorganisms. Some are true fungi, whereas the others are streptomycetes. Several species of *Penicillium* were demonstrated to produce PC, since Fleming's original experiment[2,3]. However, PCs have been reported from species of fungi other than *Penicillia*[3], such as, *Aspergillus species*[4], *Trichophyton mentagrophytes* and *Epidermophyton floccosum*[3,5,6]; *Cephalosporium species*[7,8,9,10,11,12]; *Emericellopsis species*[13,14,15,16]; *Paecilomyces persicinus*[17]; and a thermophilic fungus, *Malbranchea pulchella*[18]. A PC has also been reported from *Streptomyces*[19, 20].

Whereas, CPN compounds have been identified from a single *Cephalosporium species*[21], and from two species of *Streptomyces, S. lipmanii*, and *S. clavuligerous*[22].(After the invention of highly efficient screening methods for the beta-lactam compounds, much more variety of the antibiotic producers have been known as mentioned in Chapter III)

On the other hand, from point of view of biogenetic patterns, these two beta-lactam antibiotics behave in a different way. Penicillium-type organism is characterized by the microbial synthesis of an extensive series of PC compounds. The N-acyl side chains include any of a variety of carboxylic acid derivatives. The synthesis of specific PCs is generally in direct response to the addition of specific side-chain precursors to the culture medium, and 6-aminopenicillanic acid(6-APA) can be accumulated in the absence of side-chain precursors. However, this type of organisms is not known to synthesize CPN compounds.

By contrast, the cephalosporium-type organisms are characterized by the synthesis of a single PC with a D-alpha-aminoadipyl side chain, that is PC N[23,24,25,10]. This fermentation is insensitive to the addition of side-chain precursors and almost no 6-APA is formed.

All CPN compounds known to be formed biosynthetically possess D-alpha-aminoadipyl side-chain(except some new derivatives found recently), and

no 7-ACA(7-aminocephalosporanic acid) is formed. In fact, the discovery of CPN C was dependent on the interest in a new type of PC.

1-a) THE PENAM SERIES:

During World War II, there were two streams of developing PC industry, American and British, based on a different species of *Penicillium*. The British PC was 2-pentenyl-PC and named PC F, whereas the American PC was benzyl PC and named PC G. With regard to the fermentation of benzyl PC, it was found that the addition of the side-chain precursors not only had enhanced the specific formation of PC G at the expense of other PCs, but greatly increased the overall titer of PC[26,27,28].

In the absence of specific precursor additions, or even in a chemically defined growth medium, several PCs can be formed by *P. chrysogenum*. These are the PCs with side-chains derived from natural carboxylic acids, e.g., PCs K, F, dihydro F etc. Two unusual PC compounds accumulate during fermentation, especially if the availability of side-chain precursors in the medium is limited. These compounds are isopenicillin N or PC N[29,30], an optical isomer of the former, and 6-APA[31,32,33].

In fact, these unusual PC compounds could be the light house to guide for various semi-synthetic PCS. Sakaguchi and Murao[34] reported the microbial conversion of benzyl PC to phenylacetic acid and a substance described as "Penicin". And the latter compound was identified as 6-APA by Batchelor et al[32] and confirmed by Murao et al[35,36] later on.

Since the original reaction was discovered, its utility has been demonstrated by the many semi-synthetic PCs with excellent therapeutic value. According to the structure-activity relationships, the family members can be characterized in the following sub-groups:

A) Oral Form: Phenethicillin(PEPC), and Propicillin(PPPC)

Once allergic side-reactions were encountered in 1955, it was so serious that the oral form of PC derivatives had been recommended from point of view of safety. Phenoxymethyl PC or PC V responded immediately to such demands, since this compound was known to be rather stable to gastric acid and it was obtained easily from fermentation using phenoxyacetic acid as precursor.

However, further improvements were made by the semi-synthetic PCs based on 6-APA in terms of better bio-availability due to enhanced absorption, as well as the strengthened antibacterial activity against pathogenic organisms.

B) Anti-staphyloccocal-beta-lactamase Compounds: Methicillin(DMPPC), Oxacillin(MPIPC), Cloxacillin(MCIPC), Dicloxacillin(MDIPC), and Flucloxacillin(MFIPC)

During first few years of clinical use, PC G was extremely efficient for therapy of severe staphyloccocal infections. However, by 1950s, approximately 70% of hospital-acquired *S. aureus* infections were found to be caused by PC-resistant cultures of the organism. To counter this, such staphyloccocal infections were treated with other antibiotics like chloramphenicol, macrolide antibiotics, and Kanamycin(KM), new aminoglycoside substance,until the advent of DMPPC in 1961, since the pathogenic organisms did not possess cross-resistance against such antibiotics.

6-APA permitted the preparation of analogs with more complex 6-acylamido groups than R-CH_2-CONH- of PC G which is susceptible to hydrolysis by staphyloccocal beta-lactamase. Comparisons of the acyl groups of PCs resistant to the enzyme show that in all cases they are derived from acids in which the alpha-carbon atom is substituted with a bulky group(isoxazolyl PCs) or is contained in an aromatic ring(DMPPC). This high degree of substitution probably means that the enzyme is prevented from approaching the PC sufficiently closely to bind firmly to it and bring about its hydrolysis. Besides 5 PCs mentioned above, Quinacillin, Nafcillin, and Ancillin are known to be the same group. Oral administration can be applied for isoxazolyl PCs. A more detailed account of structure-activity relationships of this type of PCs has been written by Doyle and Nayler[37].

C) Wide-spectrum Compounds: Ampicillin(ABPC), Hetacillin(IPABPC), Cyclacillin(ACPC), Amoxicillin(AMPC), Talampicillin(TAPC), Pivmecillinam(PMPC), Bacampicillin(BAPC), and Mezlocillin(MZPC)

As pointed out already, the discovery of PC N could be the outstanding source of indications for a qualitative shift in the spectrum of susceptible organisms. PC N had only a fraction of the gram-positive activity of other natural PCs, it possessed greater activity against gram-negative organisms[38].

Remember, alpha-aminoadipic acid is owned by PC N as well as isoPC N as the side-chain moiety. Acylation of the free amino group reduced the gram-negative activity, giving the first indication of the type of function necessary for increased gram-negative activity. Later on, it was found that in a general sense, structural changes might affect cell wall permeability of gram-negative bacteria, and cause qualitative alterations of activity for PC compounds.

ABPC with the characteristic 6-N-acyl group modified by the basic nitrogen($-NH_2$), showed the dramatical change in the antibiotic spectrum(39,40). Cyclacillin keeps the similar effect by substituting phenyl group with cyclohexyl group. Further chemical modifications have been tried with regard to the NH_2 group of ABPC for improved efficiency of this wide-spectrum PC, IPABPC was proved to be of stronger activity against gram-positive organisms than the mother compound, and MZPC strengthened the spectrum against gram-negative organisms. Another modification was also made on ABPC, in order to improve the bio-availability of the drug: TAPC is a phthalidyl ester of the mother compound, and BAPC an ethoxy-carbonyloxyethyl ester. AMPC possesses p-hydroxyphenyl group in stead of phenyl of the mother compound, and this minor change gives high bio-availability of the drug due to better absorption.

PMPC is characteristic of the azepine group bound to 6-N in the form of amidine as mecillinam, as well as the esterified penicillanic acid by pivaloyloxymethyl group. This compound exhibits particularly strong activity against gram-negative phthogens. However, most of this group of PCs are susceptible to staphyloccocal beta-lactamase.

D) Anti-*Pseudomonas* Compounds: Carbenicillin(CBPC), Sulbenicillin(SBPC), Carindacillin(ICBPC), Carfecillin(PCBPC), Piperacillin(PIPC), and Ticarcillin(TIPC)

Polypeptide antibiotics like Colistin(Polymixin E) and Polymixin B, have been known to possess the powerful antibiotic activity against *Pseudomonas aeruginosa*, although they exhibit undesirable side-effects to different extent.

Pseudomonas aeruginosa was believed to be resistant against penam compounds, because of cell wall permeability as well as beta-lactamases

characteristic to this species. Certain chemically modified PCs might acquire the antibiotic spectrum against this heavily armed microorganism. Under such cicumstances, a series of new PC compounds were found to be more or less of anti-Pseudomonal activity.(cf: Fig. 1). They possess negatively charged group at the alpha-carbon of 6-N-acyl side-chain instead of positively charged NH_2 group as seen in ABPC, or its analogs. CBPC and SBPC typify the new PCs with the characteristic side-chain containing acidic function, such as carboxylic acid and sulfonic acid, respectively[41,42].

Another representative of this particular PCs is characterized by ABPC derivatives with carbamoyl or sulphamoyl group substituting free NH_2 group of the mother compound. Piperacillin exemplifies the former type of such compound.

ICBPC and PCBPC are introduced in order to enable the oral administration of CBPC, since all this type of PCs are available only in the injectable form. The alpha-carbon bound carboxylic acid of CBPC is converted into an ester form, such as indanyl or phenyl, respectively.

TIPC also possesses the carboxylic acid, binding alpha-carbon together with thienyl group instead of phenyl group of CBPC.

In general, such modified penam compounds show less anti-Pseudomonal activity than that of the aminoglycoside antibiotic like Gentamicin(GM).

1-b) THE CEPHEM SERIES:

The history of CPN compounds will tell that the successful stage has come up after a long term stagnation of research work, misdirected due to the view toward the brightful chemotherapeutic value of PC.

A search for antibiotic-producing organisms was made by Giuseppe Brotzu in Sardinia starting from 1945. He picked up a fungus which was concluded similar to *Cephalosporium acremonium*, and found that this organism produced significant amounts of antibiotic substance when grown in nutrient broth. Both culture filtrates and crude active concentrates from the *Cephalosporium species* were tested clinically in Sardinia. The results seemed to offer hopeful prospects with regard to the infections caused by both gram-positive and gram-negative pathogenic organisms. However, he gave up the attempt to go in further details and published the report concerning

the new antibiotic substance, and expressed the hope that the work would be taken up elswhere at the end of this publication.

Sir William Dunn School of Pathology at Oxford accepted his request and a culture of the antibiotic-producing organism was forwarded to Oxford in 1948. But, it was not until nearly 20 years later that the extensive developments which followed had led to products of established use in medicine.

In Oxford, the antibiotic substance, active against gram-negative as well as gram-positive bacteria was isolated and named CPN N. The first clear evidence that this substnce was a new type of PC, was obtained in 1952, and the chemical structure with a residue of D-alpha-aminoadipic acid linked through its delta-carboxyl group to the nucleus of PC molecule was suggested by Newton and Abraham[38], it was subsequently named PC N.

A second hydrophilic antibiotic was discovered among the antibiotic products of the Sardinian *Cephalosporium species* in 1953. The substance, CPN C was encountered during the chemical study of PC N, as a contaminant component, and readily obtained as a crystlline form in terms of chromatographic purification.

Despite its very low activity compared with PC N, it was resistant to hydrolysis by penicillinase from *Bacillus subtilis*[43,44]. However, the isolation of the new antibiotic in quantity appeared at that time to be a formidable undertaking. On the other hand, applications for patents with regard to the active products of the Sardinian *Cephalosporium species* of medical interst, were all assigned to the National Research Development Corporation(N.R.D.C.) established by British government. A general option for a license had been obtained from N.R.D.C. by Squibb, Eli Lilly, Merck, Pfizer, Smith Kline & French(U.S.A.), Ciba(Switzerland), Farm Italia(Italy), and Fujisawa(Japan) between 1959 and 1961, in addition to the original partner, Glaxo, U.K.

A higher yielding mutant had become available in 1957, and it enabled the experiments for the confirmation of chemical structure. In 1960, the discovery of methicillin from 6-APA and the demonstration of its therapeutic properties seemed to have prevented CPN C from clinical use. Then, a great deal depended on the discovery of a method for the production of 7-ACA on a large scale.

But, no enzyme was found to remove the sidechain from CPN C, and an ingenious chemical procedure had been discovered instead. Thus, 7-ACA became available in quantity, and an intensive study of the properties of derivatives of this compound soon led to the introduction of new CPNs.

The cephem family have the common chemical structure of four-membered beta-lactam ring fused with six-membered dihydrothiazine ring, under the chemical name of 8-oxo-5-thia-1-azabicyclo(4,2,0)-oct-2-ene. This ring system is also called 3-cephem ring which is often utilized for the nomenclature of this type of chemical compounds. For instance, 7-ACA is identical with 3-acetoxymethyl-7-amino-3-cephem-4-carboxylic acid.(Cf; Fig. 1)

The cephem compounds have been divided into three categories according to the generation in response to the selective clinical use[45]; the 1st. generation group is susceptible to hydrolysis by CPNase though stable to PCase, whereas the 2nd. generation is free of such susceptibility, and the 3rd. generation is of anti-Pseudomonal activity as well as resistant to the CPNase. Cephamycins, which are exclusively produced by Streptomyces fermentation, have kept the honorship of the 2nd. generation cephem antibiotics.

In view of the antibacterial activity as presented in Fig. 3, the 1st. generation series is characteristic of the strongest inhibition against *Staphilococci*, and the 3rd. generation signifies the outstanding inhibitions toward *H. influenza, K. pneumoniae, C. freundii, Enterobacter spp.*, in addition, less but still effective inhibitions against *S. marcescens, Ps. aeruginosa* and *B. fragilis*. The 2nd. generation is inhibitory next to the 3rd. generation toward most of gram negative pathogens except *Ps.* and *Serratia*.

A) First Generation Series:

a) Derivatives of CPN C with the chemical modification limited to the C-7 position

Cephalothin(CET) is the first CPN analog of clinical use. CPN C itself has a low order of antibacterial activity, especially less active against gram-positive bacteria than PC G. A synthetic chemical program based on modification of the CPN nucleus, 7-ACA, has yielded many new CPN antibiotics.

The generic name of CET was assigned to 7-(thiophene-2-acetoamido) cephalosporanic acid. It exhibits *in vitro* activity against both gram-positive and gram-negative bacteria, but no known activity against yeast, fungi, or viruses.

Thus, it inhibits the isolates of *Staphylococcus aureus, S. albus, Streptococcus pyogenes, Str. sp.(Viridans group), Diplococcus pneumoniae, Clostridium sp., Listeria monocytogenes, Corynebacterium diphtheria, Actinomyces sp. Bacillus subtilis, Neisseria gonorrheae, Proteus mirabilis, Klebsiella pneumoniae, Salmonella sp.*, and *Shigella sp.*, and the many strains of *Escherichia coli, Citrobacter sp.*, and *Haemophilus influenza*. However, most isolates of *Proteus morganii, Pr. vulgaris, Pseudomonas sp., Herella sp. Serratia sp., Bacteroides sp.* and *Enterobacter sp.* are unaffected by CET.

CET is not absorbed in humans after oral administration, and must be given parenterally. However, it is highly active against PCase producing stapylococci, an activity that alone would have warranted a clinical use of the antibiotic. But, pharmacological study has revealed that the biological deacylation readily took place at C-3 position through the metabolism in human tissue as well as by pathogenic microorganisms. Such metabolized compounds are proved to be less active. This also occurs in Cefapirin(CEPR), as well as Cefacetrile(CEC), because of the same functional group at C-3 position, and the degree of metabolism is believed to be ca. 20% for CET and CEPR, and only several percent for CEC, in terms of the deacetylated compound produced.

Cephaloglycin(CEG) is the first compound for oral administration, and characterized by the introduction of phenylglycine to 7-N-acyl sidechain, but already obsolete because of poor absorption as well as remarkable metabolical inactivations[46]. CEC is known to have the characteristic of being transferrable to menings among other cephem compounds, as well as of higher stability against enterobacteriaceal beta-lactamases than CET and CER.

b) Derivatives of 7-ACA with chemical modifications on both C-7 and C-3.

Cephaloridine(CER) is an analog of CET given by replacing acetoxy by pyridine at C-3, therefore, it exhibits no metabolical changes because of the substitution. However, this modification has also provided the new compound with notable side effect of nephrotoxicity.

Cefazolin(CEZ) is a unique compound characteristic of very high bio-

availability, since it is hardly metabolized in human tissues and recovered in urine at extraordinary high rate. Tetrazole, and thiazole are introduced to C-7 and C-3, respectively. After the minor change at C-3 position of CEZ, Ceftezole(CTZ) is added to the analog. This type of compounds is merited with less nephrotoxicity than CER. Other cephem compounds in this category have been developed for the purpose of oral administration. These are Cephalexin(CEX), Cefradine(CED), Cefatrizine(CFT), Cefroxadine(CXD), Cefaclor(CCL) and Cefadroxil(CFD). CEX is derived from CEG by substituting the acetoxyl group with hydrogen atom, and this compound was actually produced through ring expansion of the penam compounds, such as PC G or PC V. CEX is further modified at C-7 position by CED, replacing phenyl group by cyclohexadiene, and CED is again modified at C-3 position substituting methyl group with methoxyl group, thus CXD is obatined demanding the analog of low-toxicity. On the other hand, CCL is obtained from CEX by simple chlorination of the C-3 position, while, CFT differs from other compounds, since it possesses triazine at C-3, and para-hydroxyphenylglycine at C-7. An analog is derived by the modification of the C-3 position, and named CFD. After all, these series of cephem compounds have been accepted their specificity of the anti-staphylococcal beta-lactamase with the antibacterial spectrum represented by CET. CCL may be authorized as 2nd. generation by some authors.

B) Second Generation Series: Cefmetazole(CMZ), Cefoxitin(CFX), the cephamycin family; Cefotiam(CTM), Cefuroxime(CXM) and Cefamandole(CEM).

The discovery of the novel cephem compounds elaborated by taxonomically different microorganism, *Streptomyces spp.*, has been an epoch-making event as noted in Chapter III, since these compounds were found to possess the microbial modification at C-7 position, namely 7-alpha-methoxyl group. Therefore, such type of cephem compounds is called cephamycin with the impression of the antibiotics produced by *Streptomyces spp.* In addition, these compounds have been proved to be stable to most of the CPNase known to hydrolyze the cephem compounds. The name of the 2nd. generation is due to this particular activity. CFX is derived from cephamycin C, but CMZ is obtained by the modification of 7-ACA, introducing 7-alpha-methoxyl group chemically.

In contrast to the cephamycin compounds, the particularly modified groups at C-3 and C-7 positions lead to the compound with the stability against the beta-lactamases mentioned above. Thus, the introduction of thiazole and tetrazole to C-7 position and C-3 position, respectively, gives CTM, and the presence of the methoxyimino group at C-7 position characterizes CXM.

C) Third Generation Series: Cefsulodin(CFS), Cefoperazone(CPZ), Cefotaxime(CTX), Ceftizoxime(CZX), Cefmenoxime(CMX) and Cefotetan(CFT); Latamoxef(LMOX).

The compounds of this series can be characterized by the anti-Psudomonal activity comparable with that of CBPC, and by the strong activities against other gram-negative bacteria resistant to the 2nd. generation series. Above all, CFS exhibits the inhibition as strong as that of the aminoglycoside antibiotic against this specified organism, regardless of the poor activity against other pathogenic organisms. In general, this type of cephem compounds shows rather poor acivity against *Staphylococcus aureus*.

From point of view of chemical structure, CTX, CZX and CMX are the compounds characterized by the methoxyimino group at C-7 side-chain, whereas CFS is characteristic of the sulfon group in the side-chain.

CPZ owes its characteristic to the modified parahydroxyphenylglycine group at C-7 as well as the tetrazolemoiety at C-3 position. CEM and CFT are the newcomers in 1983, so that their evaluation will not be discussed here, but CFT is very unique, because it is an analog of cephamycin, and yet an anti-Pseudomonas compound.

LMOX is the first oxacephem antibiotic synthesized from 6-APA, with the modified side-chain groups at C-3, and C-7 as well.

All of this type of compounds are highly resistant to hydrolysis by the known beta-lactamases, since most of the enzyme-producing gram-negative organisms are sensitive to this series.

Section 2. Aminoglycoside(AGS) Antibiotics (See Fig. 2, for chemical structure)

The origin of this antibiotic family is Streptomycin(SM), discovered by Waksman et al in the cultue media of certain strains of *Streptomyces griseus*[47]. Great interest was aroused when it was found that this compound exhibited antibiotic activity against certain bacteria and particularly the organism responsible for tuberculosis. In contrast to PC, SM is the first

broad spectrum antibiotic with the high sensitivity of gram negatives as well as gram positives, especially *Staphylococcus aureus*. Therefore, in the early era of chemotherapy, it had been widely applied for the infectious diseases other than tuberculosis in Japan. However, the recognition of ototoxicity, nephrotoxicity, as well as hypersensitivity reactions in clinical use set the limitation for such wide indications.

Chemically, SM is a trisaccharide-like substance, composed of a substituted inositol, or substituted streptamine which emphasizes a chemical moiety found in other similar antibiotics as an aglycone, and a furanose and an aminosugar linked by glycosidic bonds. Therefore, this type of compounds is called the aminoglycoside antibiotics in general.

Various new aminoglycoside antibiotics have been found lateron in terms of the metabolic product by the organism of *Streptomycetes*, such as *Streptomyces spp.* and *Micromonospora spp.*, and the basic aglycone unit of these compounds is 2-deoxystreptamine(DST) in common. There are another type of disaccharide compounds based on the aminocyclitol such as Spectinomycin[48] and Fortimycin[49], as well as the cyclitol like Kasugamycin[50], but they are beyond the scope of the "Aminoglycoside Antibiotics".

The aminoglycoside family may be divided into two sub-groups in terms of the furanose unit in the molecule:

i) Presence of furanose unit: SM, Neomycin(NM) or Fradiomycin, Paromomycin(PRM), and Ribostamycin(RSM), where, SM owns the glycosidic linkage of furanose at C-4 position of aglycone, whereas other three compounds are linked to furanose at C-5 position. SM and RSM constitute a trisaccharide, but NM and PRM do a tetrasaccharide.

ii) Absence of furanose unit: Kanamycin(KM), Tobramycin(TOB), Gentamicin(GM), Micronomicin(MCR) or Sagamicin, and Sisomicin(SSM). All of this series constitute a trisaccharide.

The weak points of the aminoglycoside antibiotics will be remarkable side-effect problems as already mentioned with SM, and the antibiotic-resistant strains of pathogenic organisms under treatment. The oral administration is not available with this type of antibiotic drug, since they are not absorbed through digestive system.

A) KM Family:

KM was found by Umezawa et al[51] in 1957, and marketed in Japan soon

after the discovery of the new antibiotic(1958). Since KM has been proved to be effctive against the resistant infections developed by the pathogenic organisms which acquired resistance against PC and SM, its advantage was evaluated world-widely. Thereafterin 1965, KM-resistant strains appeared in hospital patients. The study of the mechanisms of resistance was undertaken immediately on an enzymic level in order to clarify the role of the enzymes inactivating KM.

On the other hand, the biosynthetic study of KM fermentation using a variety of mutant strains of *Streptomyces kanamycetics* led to a new KM derivative, 2'-aminodeoxy KM, or Bekanamycin. Based on this compound, 3',4'-dideoxy-bekanamycin was picked up among the analogs modified from the parent compound, based on the findings on the mode of action of the resistant organisms. The new derivative was named Dibekacin[52], and marketed in 1975 as a chemotherapeutic agent useful in treating infections of resistant bacteria including *Pseudomonas spp.*

Another new KM derivative was introduced by chemical modification of amino group at C-1 position of aglycone, from point of view of different line of analysis with regard to the resistant organisms. The substance was known as Amikacin[53], and proved to be effective against GM-resistant pathogens(1976).

Since it was found that the parent strain had produced several KM analogs in the culture media as minor components, mention should be made of Tobramycin which was isolated from Nebramycin mixtures as an active factor, and identified later as deoxybekanamycin. This compound was claimed to be active against *Pseudomonas*, as well as KM-resistant organisms.

With regard to the development of such modifications, further details will be given in Chapter III.

B) GM Family:

GM is a metabolic product of *Micromonospora sp.* with the characteristic deoxyaminosugar linked glycosidically to C-4 position of DST. It was found that this new aminoglycoside antibiotic was active against KM-resistant pathogenic strains obtained from clinical isolates, in addition, it exhibited the anti-Pseudomonal activity[54,55].

The discovery of this novel antibiotic substance had enhanced a great deal the chemical modification studies on KM which was produced by *Streptomyces sp.* This fact will be discussed later in Chapter III. GM is actually a mixture of three components, such as GM C_1, GM C_{1a} and GM C_2, whereas Micronomicin, or Sagamicin[56], is a new GM family member with a single component. Sisomicin[57] is also one of this family produced by a different species of *Micromonospora* and found to be dehydro-GM C_{1a}.

C) Paromomycin Family:

Paromomycin is closely related to the old antibiotic, NM, constituting a tetrasaccharide and found in 1958[58]. This substance had been discovered by various research people in different institutes so that the discoverers gave the different generic names independently, such as "aminosidine", "paromomycin", "catenulin", "hydroxymycin" and "zygomycin A", since different *Streptomyces species* were claimed to produce the new antibiotic substance. However, these antibiotic substances were identified as a single compound with the same chemical structure in 1963. Such a confusing topic may represent a dynamic aspect of competitive antibiotic research performance. This type of antibiotics is mainly utilized orally for disinfecting the intestinal tract in current Japanese chemotherapy.

D) Ribostamycin(RSM):

This antibiotic substance is a trisaccharide compound containing an isolated ribose unit and its chemical structure seems to relate to NM B, except the second aminosugar is glycosidically linked to the ribose in the case of NM. RSM was found by Japanese research institute[59] in 1970, but the biosynthetic study carried out by Baud et al, using a mutant strain of *Streptomyces fradiae* , a NM-producing organism, revealed that RSM had been accumulated in the culture medium instead of NM[60]. The fact may conform to the suggestion that the second aminosugar moiety of NM might have been blocked in the biosynthetic pathways to build up NM.

Recently, summarized reviews have been published by various authors[61] on the biogenetic studies of aminoglycoside antibiotics.

CHAPTER III: BACKGROUND OF PROGRESS IN THE ANTIBIOTIC INDUSTRY

The prosperity of the modern antibiotic industry has been brought about by the sequence of discovery of the novel substances developed by the two different categories of technology;

1) those antibiotics occurring naturally, based on the particular method of screening for the antibiotic-producing microorganisms,
2) the novel compounds derived from the parent antibiotic in terms of the chemical modification leading to more potent structure. In the latter, the method of modification may depend on the chemical reaction, or the microbial conversion, and both are mixed in some cases.

Since the starting substance was obtained naturally in general, the novel antibiotics derived from the chemical modification have been called the semi-synthetic compounds.

In most cases, microbial reactions do not give the substance in a single component; one strain of certain species of microorganism will give a series of analogs, or conversely, the same analog of an antibiotic substance may be elaborated by the strains of taxonomically different species, or sometimes different genus. Mutant strains may also give the modified structure of the parent antibiotic.

The first example of the chemically modified antibiotic was found in the tetracycline(TC) family compounds in achievement of enhanced antibacterial activity.

Then, the inactivation of antibiotic substances has come to be inevitable due to the appearance of antibiotic-resistant strains of the pathogenic organisms. Fortunately, an intensive study on the basis of molar biology and biochemistry for analyzing the mechanisms of resistence caused by the resistant pathogens, has revealed the modified antibiotic structures which prevent from the inactivation by blocking the site susceptible to the enzymatic reaction. In addition, structural studies on certain naturally occurring antibiotic substance have suggested the possibility of more potent analogous compounds due to modification. A typical example can be seen in the beta-lactam antibiotics as well as the aminoglycoside antibiotics.

On the other hand, the elucidation of the mechanisms of antibacterial action has been achieved as well, due to the integrated efforts made by microbiologists and biochemists. Molar genetic studies on the gram-negative pathogens, particularly *Escherichia coli*, have led to an efficient method of screening toward various types of novel beta-lactam antibiotics. Using this tool, more scientific approach for the desired novel compound has been established.

Section 1. Chemical Modification of the Structure of Parent Antibiotics

With regard to the chemical modification process, an epoch-making approach has been first demonstrated successfully with the chemical synthesis of cortisone using a delicate microbial reaction. It was found that the progesterone added to the culture medium of *Rhizopus nigricans* had been converted to 11-alpha-hydroxyprogesterone, an intermediate compound readily convertible to cortisone[62]. This reaction was due to the selective oxidation of the specified position of the substrate elaborated by an enzyme. This event is very important in suggesting that a microbial reaction might be incorporated to a certain type of synthetic chemical reaction as a unit process giving more efficient sequence of reactions. For instance, using such a chemico-enzymatic process[63], PCs have been readily converted into 6-APA by means of PC-acylases, or conversely, several cephem compounds have been synthesized using the precursor substance as substrate.

On the other hand, various studies on the relationships between chemical structure and biological activity have developed the chemical modifications of the parent antibiotic in order to arrive at the derivatives of more potent drug value. In 1953, Sheehan, best known of the total synthesis of PC, suggested the following drug design for probable PC compound[64]:

1. acid stability
2. broadened microbiological spectrum
3. activity against resistant organisms
4. less allergenicity
5. greater metabolic efficiency(better oral absorption, slower excretion)

Most of the requirements are now fulfilled in terms of penam- and cephem-compounds developed later on.

Another example of successfully modified antibiotic has been demonstrated with the aminoglycoside antibiotics by Umezawa et al who made the

theoritcal approach based on the biochemical analysis on the mechanisms of resistance caused by KM-resistant organisms since 1966.

Here, a review will be given on the particular antibiotics such as TC, beta-lactams and aminoglycosides.

A) Tetracyclines(TC):

TCs are known to have notable antibacterial activity against a broad range of pathogenic microorganisms including many gram-negative and gram-positive bacteria, species of *Rickettsia* and *Mycoplasma*, certain *Protozoa* and large *Viruses*, therefore, they are called the prototype of the broad spectrum antibiotics. Because of high affinity to the heavy metals such as Ca, Fe and Mg, forming chelate compound, TCs are not the drug of choice for pregnant patients. Nowadays, the resistant pathogens widely spread in modern therapy also limit the usefulness of this type of antibiotics, but they are still in a position of first choice for *Vibrio spp.* like *V. comma and V. parahaemolyticus, Brucella, Rickettsia, Mycoplasma* and *Chlamydia*. Their mode of antibacterial action is known to be the inhibition of bacterial protein synthesis, but the action on mammalian ribosomes is thought to be almost negligible because of little permeability to the cells.

Chemically, TCs are unique in the structure based on the naphthacenic carbon skelton as shown in Fig. 4. Later on, the second naphthacenic antibiotics were also reported in the form of aminosugar glycoside[65]. These are known to possess the oncostatic activity, and produced by various strains of genus *Streptomyces*. They are called anthracyclines.

The following TC compounds are known to occur naturally; chlortetracycline(CTC) by *Streptomyces aureofaciens* in 1948[66], oxytetracycline(OTC) by *S. rimosus* in 1950[67], tetracycline(TC) by *S. viridifaciens* as well as *S. aureofaciens* in 1954[68], and demethylchlortetracycline(DMTC) by a variant of *S. aureofaciens* in 1957[69].

In view of similarity of chemical structure of this type of compounds based on the naphthacene skelton, the nuclear compound is named TC.

TC was first derived from CTC by dechlorination in terms of hydrogenation[70], so that this compound should be considered the origin of the chemically modified antibiotic.

It is noteworthy that the same antibiotic compound was also elaborated by the microorganisms. This finding suggested the possibility of discovering new analogs of an antibiotic substance in terms of selecting mutant strains of the antibiotic producer. Similarly, DMTC exhibited the intensified strength in antibiotic spectrum due to the elimination of alpha-6-methyl group, suggesting the possible chemical modification by microbial reaction.

The next example was the roly-TC whose site of substitution was carboxamide at C-2 position where pyrrolidine was introduced to modify the N-atom[71]. High water solubility was confirmed with this derivative, and Roli-TC nitrate was evaluated as an acceptable injectable form.

Historically, the chlorinated compound, like CTC and DMTC, was found to be considerably more active than others against a variety of *Staphylococci, Streptococci* and *Pneumococci*[72,73,74,75,76], and coliforms[77]. The discoverer of OTC, Pfizer research laboratories, had been distrubed by this fact, and concentrated their efforts toward the better derivatives in terms of modification of OTC. The new compound obtained thus is Methacycline[78]. This substance is characterized with the 6-methylene group caused by dehydration, showing higher bio-availability than DMTC on oral administration. Methacycline was readily converted to another derivative[79] by hydrogenation of the methylene group, corresponding to alpha-6-deoxy-OTC under the generic name of Doxycycline. Better bio-availability was also confirmed in clinical use.

Another novel TC derivative was developed independently by Lederle group, with the characteristic of 7-dimethylamino-6-demethyl-6-deoxy-TC. Minocycline is the generic name of this substance. It differs from other TCs in antibacterial behavior, since this substance is active against TC-resistant *Staphylococci* as well as *Mucobacteria*[80].

The mechanism of bacterial resistance against TCs was found to be due to the lowered cell permeability of the antibiotic.[81] Chemically modified derivatives like Doxycycline, or Minocycline, possess an enhanced permeability against gram-positive organisms, but does not against gram-negative organisms. In addition, the increased permea-

bility to the mammalian cells is also inevitable. Thus, sometimes the modified compound requires the precaution of side-effect.

Up to the moment, no further example has been reported with regard to the new TC derivatives. The structure-activity relationships seems to rely on the functional group at 5,6 and 7 positions of the carbon skelton, respectively.

B) Beta-lactam Antibiotics:

Because of the delayed introduction of the cephem compounds, the problems of the resistant strains of pathogenic organisms have begun with the penam compounds. PC G derivatives were first encountered the key problem brought about by very poor bio-availability on oral administration, because of its destruction in gastric fluid. Later chemical study on the cleavage of the beta-lactam ring revealed that the sensitivity of PC to acid parallels the ease of formation of oxazolone which subsequently is transformed to the penillic acid. Formation of penillic acid has been minimized by introducing the electron-withdrawing group at the N-acyl side-chain, because the nucleophilicity of the N-atom in the ring system is weakened by this effect. The discovery of the phenoxymethyl-PC, or PC V, which was obtained through *Penicillium* fermentation using phenoxyacetic acid as precursor, is the first step toward the acid-resistant oral form of PC. Based on 6-APA, further effective derivatives are achieved taking advantage of introducing much more efficient substituents to the N-acyl side-chain than that of PC V, since the microbial incorporation is not available other than phenoxyacetic acid. These substances are known as PEPC and PPPC with the functional groups of phenoxyethyl and phenoxypropyl, respectively. As mentioned in Chapter II, the 6-APA had been found in PC fermentation as a minor component in the early stage. After then, a variety of microorganisms have been known to produce PC-acylases which split off the acyl moiety, subsequently leaving the amino function at C-6 position. These findings immediately bind to the mass production of 6-APA, and enable the introduction of a wide-range synthetic PCs. The same is true with 7-ACA, but this product has been exclusively due to chemical conversion, since the nature of of 7-N-acyl function is characterized by D-alpha-amino-

adipic acid, which has no affinity to deacylating enzymes(the presence of such enzymes is now known, but their industrial use is not known). However, a quite many cephem compounds are enzymatically synthesized using corresponding precursor acids together with 7-ACA. In Fig. 5, several cases of such example are summarized.

Semi-synthetic PCs, such as ABPC and MPIPC are also relatively stable to acid, again because of the electron-withdrawing substituent in the N-acyl side-chain.

Compared with the penam compounds, the cephem compounds are relatively insensitive to acid regardless of the N-acyl side-chain, because of the intrinsically poor nucleophilicity of the N-atom in the dihydrothiazine ring(82).

As exemplified above, the chemical instability of the beta-lactam ring of the penam compounds causes the susceptibility to a wide variety of cleavage reactions. For instance, PCases hydrolyze PC into an inactive penicilloic acid(83), the hydrolytic reaction will occur in alkaline conditions as well, and the analogous penicilloic acids are produced from all PCs regardless of the nature of 6-acylamino group, and with all the beta-lactamases regardless of their source or detailed characteristics. According to the study of the mechanisms of antibacterial action, PC molecule is hydrolyzed to penicilloate and taken up by the transpeptidase in stead of D-alanine which should be removed from D-alanine-D-alanine linkage located in the pentapeptide during the course of cell wall synthesis. In these circumstances, the transpeptidase may be looked upon as a special type of beta-lactamase. Penicilloic acid may react with tissue protein to form protein-penicilloyl complex with the function of allergic side-reaction.

Another degradation product of PC can be the penicillenic acid which has been implicated as a factor in PC allergy as well, since the penicillenate is thought to react with the amino groups of protein to form haptens of antigens. More or less, the same situation will be involved with the cephem compounds to some extent.

Much of the chemically modified compounds of the beta-lactam antibiotics have been presented in Chapter II, and here, a review will be made in consideration of the structure-activity relationships among these particular compounds. First, we will analyze the effect of the beta-lactamases by which the C-N bond in the beta-lactam ring of PC or CPN is splitted off. This type of enzymes have been known to be widely distributed among bacteria, and found in *Staphylococcus aureus, S. epidermidis, Bacillus cereus, B. licheniformis,* and various *Mycobacterium spp.* among gram-positive organisms; strains of *Escherichia coli, Proteus spp., Klebsiella aerogenes, Enterobacter cloacae, Pseudomonas aeruginosa,* and other gram-negative organisms also possess such enzymes.

In many instances, there is a strong correlation between the resistance of an organism to a beta-lactam antibiotic and its ability to elaborate a beta-lactamase capable to destroy the antibiotic. It was also found that some bacteria could make more than one type of enzyme.

PC is transformed to penicilloic acid by the enzymic hydrolysis as mentioned above. PC has one acidic group, whereas the corresponding penicilloic acid has two strong acidic functions.

The situation with the CPNs is more complicated by the presence of two sites in the molecule which can be modified chemically, and changes in both side-chains give compounds with altered enzyme susceptibility and changed antibacterial properties. In general, the cephem compounds are not sensitive to staphylococcal beta-lactamases, regardless of the sites of modification.

Certain systematic classification may be available with regard to the known beta-lactamases based on the suggestion by Richmond et al[84] as shown in Table 6.

Essential to an understanding of the events through which an antibiotic exerts its action is a familiarity with physical and chemical characteristics of the bacterial cell. Studies of cell wall composition have shown that, in bacteria, gross differences exist between those organisms which give a Gram-positive stain and those which are classified as Gram-negative.

The Gram-negative group have a cell wall composition high in protein and lipid and low in reducing sugar and hexosamine content, when compared with Gram-positive bacteria. It is evident that response to the Gram staining technique is related to chemical composition of the cell wall.

The lipophilic nature of the cell wall of Gram-negative bacteria causes the problems of permeability barriers which will put the behavior of either the beta-lactamase leaving out, or the antibiotic coming in, under control. This is a very important point when we deal with the topic of the inactivation of antibiotic activity caused by the Gram-negative bacteria.

1) The beta-lactamases from Gram-positive bacteria:

A great many strains of *Staphylococcus aureus* can destroy large amounts of PC G by means of beta-lactamase activity, and Richmond[85] found that these organisms can make three distinct but similar enzymes. PCs with the general structure R-CH_2-CONH- as the 6-acylamido group are all susceptible to hydrolysis by staphylococcal beta-lactamase. When the alpha-carbon atom is substituted with a bulky group like the phenyl-isoxazolyl, or is contained in an aromatic ring such as dimethoxyphenyl, the corresponding PCs are resistant to the enzyme. This high degree of substitution may build up the steric hindrance preventing the enzyme from approaching the PC, and the resistant PCs have a very low affinity for the enzyme. If the degree of steric hindrance is not adequate due to only a small substituent at the alpha-carbon, the corresponding PCs, such as ABPC and CBPC, are still susceptible to the enzymatic hydrolysis.

On the other hand, the same steric effect on the sensitivity of the substrate to hydrolyze may be applied to the transpeptidase as well, therefore, the beta-lactamase-resistant PCs show far less activity against the nonenzyme-producing strains.

Cephem compounds, such as CEZ, CEC and CEPR having simply substituted acetic acids as 7-acyl moiety, are almost completely stable against the staphylococcal beta-lactamases. However, recent reports have pointed out the presence of *Staphylococcus aureus* or *Streptococcus pneumoniae* resistant to the enzyme-resistant PCs and CPNs, through alteration of

cell wall synthesizing system[86]. This is due to the genetic response other than beta-lactamase production.

It is also known that *Mycobacterium spp.* like *M. tuberculosis*, or *M. smegmatis* also produce certain type of the enzyme which inactivates PC G, although the role of this enzyme in mycobacterial resistance has not been established in relation to the permeability barrier which also exists in these organisms. PCs and CPNs with nonsterically hindered group are, in general, sensitive to the enzymes from all species of *Mycobacteria*.

2) The beta-lactamases from Gram-negative bacteria:

As seen in Table 6, the enzyme elaborated by the Gram-positive organisms is an untransferrable PCase which is mediated by plasmids, and belongs to an exoenzyme, in general. This type of PCase is inducible in the presence of PCs as well as CPNs, and has little effect on CPN C.

On the other hand, there have been numerous reports of beta-lactamase activity in many species of Gram-negative organisms, with the picture much more complex than that of the Gram-positives, since certain organisms such as *Enterobacter, Serratia, Hafnia, Iseudomonas,* and *Iroteus morganii* are knwon to produce consistently the enzyme which selectively destroys certain CPN derivatives, therefore, they are resistant to such CPN compounds. In addition, *Shigella, Escherichia coli, Iroteus vulgaris* and *Iroteus rettgeri* also produce CPNase in some cases. Thus, the beta-lactamases elaborated by the Gram-negatives are in variety and all are the perienzymes which are subdivisible into the two different categories according to their genetic origin;

a) PCases of Type III and Type V, mediated by transferrable R-plasmids, in consequence, the same kind of beta-lactamase activity can be found in widely different organisms. In this case, the genetic substance is transferred from resistant strains to sensitive strains via transduction in terms of parasitic viruses or bacteriophages, as well as conjugation between resistant and sensitive strains.

This type of PCases may act on CER, or MPIPC.

b) PCases of Type II and Type IV, as well as CPNase, mediated by chromosomes, therefore, species-specific. This type of PCases acts on CER as well, and the CPNase attacks the compounds of 1st. generation series of the cephem compounds.

Most of this type of enzymes are not inducible except some which require extraordinally high concentration of inducer used, for the maximal enzyme production. If sufficiently large concentrations of inducer are used, other Gram-negative species may produce an inducible enzyme.

Some Gram-negative bacteria possess a permeability barrier which may act to make the organism more resistant, because the antibiotic is kept away from the site at which it acts. On the other hand, such a barrier may prevent the antibiotic reaching the cellular location of the beta-lactamase, and so prevent its rapid degradation.

An enzymic component in the cell wall to which a PC or CPN binds and from which it can't be recovered intact, may also be regarded as a special type of beta-lactamase. This type of enzyme, however, is different in not being able to bring about the rapid destruction of large amounts of substrate which can be observed with the more obvious beta-lactamases.

As mentioned above, *Enterobacter, Citrobacter,* indole-positive *Proteus* are not sensitive to most of the cephem compounds(1st-, and 2nd-generation series), since either they can produce CPNase or they have a permeability barrier. Therefore, those cephem compounds having the sensitivity toward these specified organisms are called the 3rd generation series, since they show the antibiotic activity by overwhelming the problems of beta-lactamases and permeability barrier.

Pseudomonas aeruginosa is the organism heavily armed with permeability barrier as well as beta-lactamase activity. In this regard, CFS is a unique substance to challenge this organism, since the substance has a high permeability to the cell wall and shows resistance to lactamases elaborated by the organism, though it is of poor activity against other organisms.

The beta-lactamase activity has been discussed *in vitro* as well as *in vivo*, in the former case, much of the classical biochemical investigation of the beta-lactamases using purified enzymes and substrate concentrations too high to be therapeutically feasible, may have little relevance to what actually happens clinically when a crude enzyme in an intact living cell encounters comparatively low substrate concentrations. In order to express the functional efficiency of the enzyme under these conditions, the concept of "physiological efficiency" was introduced[88]. It is defined as the specific activity(micromoles of substrate metabolized per microgram of enzyme protein per hour) at enzyme saturation, divided by Km(molarity) for any substrate. In the category of greater value of Km, that means, the enzyme has low affinity with the substrate, the efficiency of the enzyme depends on the amounts produced. A typical example will be the R-plasmids mediated PCases which show hydrolytic activity against CER in most cases, but sometimes not.

Cell wall permeability appears to be partly responsible for the relative amounts of Gram-positive and Gram-negative activity of PCs and CPNs. In a general sense, structural changes which may affect permeability appear to cause similar qualitative alterations of activity for both the CPNs and PCs. Permeability appears to be mediated by the side-chain polarity. The ability of various beta-lactam antibiotics to exhibit a qualitative shift in the spectrum of organisms inhibited was first realized with the discovery of PC N, subsequently followed by CPN C. In view of the antibiotic activity, both PC N and CPN C have less than 1% of the activity of PC G against a PC-sensitive staphylococcus[89], but they show superior activity against Gram-negative organisms.

The side-chain moiety of PC N suggests more hydrophilic nature of this compound than other types of the natural PCs.

An analysis of relative antibiotic activities toward Gram-positive and Gram-negative bacteria expressed in terms of the lipophylic character of the beta-lactam antibiotic has appeared[90]. It is found that PCs and CPNs which are most active against *Escherichia coli* are more hydrophilic than those which are active against *Stapnylococcus aureus*.

Their results are interpreted in terms of variation in lipid content of the cell wall. The *E. coli* has a greater lipid content and can, therefore, bind and prevent penetration by lipophylic compounds. It was found that Gram-positive organisms grown under conditions which increased cellular lipid content developed increased resistance to PCs[91].

Thus, comparison of the activities of benzyl-PC with D-alpha-amino-benzyl-PC shows this effect dramatically. If a carboxyl group(CBPC) or other acidic function(SBPC) is introduced in place of the amino group, the resulting compounds are much less active against Gram-positive bacteria, and show a change in Gram-negative spectrum as exemplified by their inhibition of indole-positive *Proteus sp.* and *Pseudomonas*[92].

Thus, in the penam series, changes in 6-aminoacyl substituent bring about dramatic changes in resistance to beta-lactamase as well as in antimicrobial spectrum toward Gram-negative bacteria. Such particular properties appear to depend wholly on the configuration of this substituent.

In the case of cephem compounds, the ring system must also greatly influence the enzymic hydrolysis, since the characteristic nature of CPN C has been realized by the resistancy against staphylococcal PCase.

However, the cephem compound which exhibits antibiotic activity against CPNase-producing Gram-negative organisms has first come to the light in the form of the 7-alpha-methoxy-CPN C produced by a strain of *Streptomyces limpianii* which has been reported by Waksman and Henrici[93]. Another streptomycete species, such as *S. clavuligerus* also yielded new CPN-related compounds. Both cultures also produced PC N, but the first reported instance of beta-lactam production by a stereptomycete was the isolation of PC N in the Merck laboratories, though no further investigations followed[94].

Actinomycetes are generally sensitive to beta-lactam antibiotic[95], therefore, species of *Streptomyces* that produce beta-lactam antibiotics are anomalous for their sensitivity to these antibiotics.

These particular CPN-related compounds are called cephamycin, in response to the taxonomical difference of the antibiotic producing organisms.

The new type of the cephem compounds was proved to be resistant CPNases produced by Gram-negative organisms, such as *Serratia*, indole-positive *Proteus*, and *Bacteroides*, etc.

It is said that the discovery of this unique substance was due to the success of novel screening techniques using the hypersensitive strains for beta-lactam antibiotics obtained by mutagenesis of certain Gram-negative bacteria as test organism. The cultures mentioned above were isolated from soil samples collected in South America which gave 1,852 cultures composed mainly of *Streptomyces spp*.

In the alpha-aminoadipic acid-containing antibiotics, the presence of a methoxyl function at C-7 in stead of hydrogen atom, results in diminished activity against the Gram-positive microorganisms. In contrast, a significant increase in inhibitory properties toward Gram-negative bacteria is observed. This shift in antimicrobial spectrum appears to be consistent with the correlations concerning the differences in lipophilic character of the cell walls of various bacteria.

Increased permeability may also influence the susceptibility to hydrolysis due to CPNases. Thus, cephamycin derivatives have given rise to the 2nd. generation series of cephem compounds.

Recently, a new type of naturally occurring cephamycin has been reported with the characteristic functional group of 1-methyl-1H-tetrazole bound to C-3 position via thiomethyl group, since the antibiotic-producing strain(*Streptomyces oganoensis)* can take up this substance added as a precursor compound[96].

On the other hand, CPNs occurring naturally contain 3-acetoxymethyl-3-cephem nucleus, and the acetoxyl group in this system has shown a surprising tendency to be displaced by nucleophiles, leading to a host of 3-(substituted)methyl-3-cephem nuclei.

A variety of substitutes by nucleophilic displacements have been analyzed in relation to antibacterial activity, among which 3-carbamoyl-methyl-3-cephem compound was found in Cephamycin C. It is now accepted that 3-substitutes obtained by nucleophilic displacements with hetero-aromatic thiols or pyridines will give a potent cephem compounds in association with the functions at the C-7 position.

CTM is a typical example, since aminothiazole moiety at C-7 in combination with tetrazole thiol group at C-3 position makes up the resultant cephem compound of high cell wall permeability. This factor gives rise to antibacterial activity against certain Gram-negative bacteria, such as *Klebsiella, Enterobacter, Citrobacter* and indole-positive *Proteus*. The same, or rather stronger effect is also demonstrated by CXM which has the methoxyimino function at C-7 position.

The presence of 7-alpha-methoxyl group or 7-beta-methoxyimino function seems to play an important role in the insensitivity to CPNase presented by the resultant compounds. Thus, CTX, CZX and CMX are developed based on aminothiazole moiety and methoxyimino function at C-7 position with different substituents at C-3 position. All of this group show strong antibacterial activities against CPNase-producing Gram-negative organisms including *Pseudomonas aeruginosa* partly due to the insensitivity toward such CPNases, and have got the ranking of the 3rd. generation series.

The substitution of 7-alpha-free amino group by carbamoyl derivative with the introduction of dioxopiperadine is also successful to result in the cephem compound of the category of the 3rd. generation. This is the case of CPZ.

Several novel cephem compounds under clinical trials are known to be characterized either with 7-alpha-methoxy-3-cephem nucleus, or the 7-beta-methoxyimino function including its related substitutes.

Finally, mention should be made of Latamoxef, a typical synthetic oxacephem compound, since the substance has been derived under the drug design based on the knowledge of the structure-activity relationships so far accumulated. The invention is due to the research activity of Shionogi Pharmaceutical Industry Co. in Japan. As seen in Fig. 1, the structure-activity relationships are as follows:

a) oxygen atom at 1-position in place of sulphur: enhancement in antibacterial activity particularly against Gram-negative bacteria, mainly due to the increased cell wall permeability.

b) methoxyl function at 7-alpha-C position: insensitivity against beta-lactamase Type I(CPNase) mentioned in Table 6.

c) carboxyl function introduced at the alpha-carbon atom of 7-beta-side-chain moiety: anti-pseudomonal activity.

d) p-hydroxyl function introduced to the terminal of 7-beta-side-chain moiety: enhancement of bio-availability toward rapid absorption.

e) tetrazole thiol substitute at C-3 position: protection from tissue or microbial metabolism, as well as enhancement of anti-Gram-negative activity.

Remarks: With regard to the beta-lactam antibiotics, the degree of sensitivity to beta-lactamases is used to be expressed not in absolute value, but in relative values in relation to those obtained for the compounds of reference standard, such as PC G, ABPC or MPIPC, or CER, CXM, etc. according to the conditions concerned.

In most cases, the 2nd. generation series show the degree of enzymatic hydrolysis corresponding to the values less than 1% of those of the 1st. generation series like CER.

C) Aminoglycoside(AGS) Antibiotics:

This kind of antibiotics is known to have the mechanism of broad antibacterial action based on the inhibition of protein synthesis of bacterial cells, since it has been shown both *in vitro* and *in vivo* that SM, by interacting with ribosome, causes misreading of the genetic code and refers to the control of the fidelity of translation of genetic information.

Animal cells are relatively insensitive to SM[97], typical representative substance of the AGS antibiotics, although in clinical use ototoxicity, nephrotoxicity and hypersensitivity reactions have been recognized. Other chemically related compounds of this family possess more or less the similar function to SM.

Streptomyces spp. are used to be the sole producer of this type of antibiotic in the early stage, but genus *Micromonospora* has been introduced to give the similar type of antibiotics, and now *Bacillus sp.* is also known to elaborate analogous substances like Butirosin[98].

Similar metabolic pathways seem to be utilized by these microorganisms of different source in the manner of forming the paronamine or the neamine as an intermediate compound; when 2-amino-2-deoxy-hexose is glycosidically linked to 2-deoxystreptamine(DST) as aglycone, the resultant disaccharide is the paronamine, whereas if the sugar is 2,6-diamino-dideoxy-hexose, then the neamine is formed as the disaccharide compound. PRM will be derived from paronamine, and RSM, NM, TOB, GM and BTS(butirosin) will be produced via neamine.

In Fig. 6, probable biosynthetic pathways[99] are presented with regard to the formation of AGS antibiotics by *Streptomyces* as well as *Bacillus*. *B. circulans*, producer of particular substance BTS, exhibits the function to modify the amino group at C-1 position of DST by introduction of L-gamma-amino-alpha-hydroxybutyric acid(AHB) moiety, whereas *Streptomyces* does not. The mutant strain of the bacillus devoid of such modifying function has been known to accumulate RSM[100], since further metabolic pathways to BTS are blocked. It was also found that BTS molecule was free of enzymatic inactivation by phosphorization

caused by the RSM-resistant organisms, regardless of the presence of hydroxyl group at C-3' position.

The reported findings have been reconfirmed immediately by Japanese research organization, and interpreted to the different type of AGS compound, KM A. Thus, KM A-1-N-AHB[101] was synthesized and named Amikacin. It may be assumed that the steric hindrance caused by substitution will prevent the approach of the inactivating enzyme from the site of its target. The same idea is also applied to SSM, leading to 1-N-ethyl-SSM, called Netilmicin[102].

Thus, BTS, naturally occurring unique AGS derivative plays an important role against the mechanisms of biological inactivation by the resistant organisms.

Basically, the metabolic pathways of antibiotics of this kind are closely related to the cell wall synthetic pathways of the antibiotic-producing organisms, since both pathways are dependent on D-glucosamine as a common intermediate substance to go through the metabolism. For instance, it was reported that cell walls of *Streptomyces fradiae* contain inordinately large amount of glucosamine[103].

Normally, cell wall synthesis is carried out by taking up glucosamine, but when this metabolic pathways are put under control by certain control mechanisms, the glucosamine may shunt toward the antibiotic pathways.

In fact, it was found that the addition of PC to the culture medium of *S. griseus* gave rise to the enhancement of the production of SM[104]. In general, the inhibitors for cell wall synthesis, such as PC, bacitracin, cycloserine, etc., added to the culture medium of *Streptomyces spp.* as well as *Micromonospora sp.* are known to support the shunting of glucosamine toward the antibiotic production.

Inversely, under certain conditions, the antibiotic produced may undergo inactivating reactions caused by the control mechanisms of the producing organism, for example, 6'-N-acetyl KM was found in the fermentation broth of *S. kanamyceticus* as an inactivated form of KM due to 6'-N-acetyltransferase elaborated by the organism. However, the same organism also produced 6'-N-acetylKM-amidohydrodase[105] by which KM can be restored from the inactivated form.

In many other cases, the presence of inactivated form of this type of antibiotics in terms of N-acetylation or O-phosphorization has been detected in the culture medium in conformity with the presence of such inactivating enzymes in the antibiotic-producing organisms. However, the O-nucleotidyl derivatives resulting from O-nucleotidyltransferase are not known yet in the fermentation medium.

Gram-negative and Gram-positive pathogenic clinical isolates exhibiting resistancy to AGS antibiotics also possess the function of inactivating the antibiotics in terms of N-acetylation as well as O-phosphorylation or O-nucleotidylation. A summary of such enzymes is presented in Table 7.

GM which is elaborated by the genus *Micromonospora* was first known to have the antibacterial activity against *Pseudomonas spp.* as well as KM-resistant strains of Gram-negative organisms. Later biochemical study has revealed that the activity against the particular organism, *Pseudomonas*, seems to depend on the absence of the hydroxyl group at C-3' position. Similarly, *Streptomyces sp.* also produces 3'-deoxy-AGS compound like TOB with the antibiotic activity against this organism.

On the other hand, one biochemical basis of chloramphenicol(CP) resistance has been demonstrated to be due to the presence of CP-inactivating enzymes in cell-free extracts of an episome-carrying strain of *E. coli*[106]. This enzymatic inactivation of the antibiotic required the presence of acetyl-Co A, and the similar inactivation was demonstrated on KM as well. The Institute of Microbial Chemistry founded in 1962 by Dr. Umezawa, also opened the research area to clarify the mechanism of bacterial resistance to KM as well as other AGS antibiotics on the basis of biochemistry. Enzymes inactivating AGS antibiotics were found in disrupted cell of typical resistant strains of *E. coli* carrying R-factors obtained from clinical sources. The reaction products of these enzymes were isolated and the structures were elucidated. An intensive study laid on this research line has revealed the role of various enzymes inactivating the antibiotics as follows:

a) AGS 3'-phosphotransferases, or APH(3'), I, II and III, catalyzing the transfer of the terminal phosphate of ATP to the 3'-hydroxyl group of the antibiotic molecule. This type of resistant organisms is

inhibited by 3'-deoxy-, or 3',4'-dideoxy-derivatives of KM, RSM and BTS, respectively. Whereas, GM is intrinsically free of inactivation caused by such enzymes.

b) AGS 2"-nucleotidyltransferase, or AAD(2"), catalyzing the reaction of ATP,GTP or ITP to the 2"-hydroxyl group of the substrate molecule. Acylation of the 1-amino group with L-4-amino-2-hydroxybutyric acid, L-5-amino-2-hydroxy-n-valeric acid, or L-, or D-isoserine gives the derivatives which inhibit the growth of resistant organisms producing the inactivation enzyme. In addition, regardless of the presence of 3'-hydroxyl group, these derivatives have been found not to undergo the reaction of APH(3') I, and inhibit this type of resistant organisms. The location of 2"-hydroxyl group neighbouring upon that of the 1-amino group is indicated by the molecular model of KM, but the additional protection against APH(3') has not been known until the discovery of BTS.

c) AGS 6'-acetyltransferase, or AAC(6'), catalyzing the reaction of acetyl-Co A by transfer of the acetyl group to 6'-amino group. Methylation of the amino group will give active compounds against this type of resistant strain. 3-N-acetyltransferase was also confirmed in *E. coli* against GM C.

Details of above mentioned intensive study on AGS antibiotics have been summarized in the reviews given by Drs. Umezawa brothers[107,108].

Finally, mention should be made of "mutational biosynthesis"[109], since this technology has been developed to create novel AGS substances taking advantage of the fact that the enzymes in the secondary metabolism are not substrate specific. For instance, the blocked mutant of *Streptomyces fradiae* devoid of the pathways regarding DST formation could be created through genetic manipulation, and the novel type of NM analogs was elaborated by taking up analogs of DST, such as streptamine. At this moment, no industrial application of this technique has been successful, but the novel compounds with increased antibacterial activity or resistance against inactivating enzymes may be derived from such microbial reactions in future.

Section 2. Establishment of Screening Process for the Selection of Micro-organisms which may elaborate Novel Beta-lactam Antibiotics as well as Beta-lactamase Inhibitors

The discovery of a new antibiotic primarily depends upon the micro-organisms which will elaborate the antibiotic substance. The method of screening for such specified organisms has been the key technology essential to arriving at a new antibiotic.

Microorganisms are known to be distributed widely and profoundly among natural environment, such as soil, river, lake, sea, or plants and animals, etc. For instance, it is well confirmed that there are around 10^9 organisms in 1g of soil[110].

In the very early stage, PC was found to be produced in the culture plate of *Staphylococcus aureus* by certain species of *Penicillium*, which had been brought about by chance of contamination in the form of airborne spores.

On the other hand, the CPN-producing organism was isolated from the microbial flora of sea water collected near sewage outlet under the prospect that the process of self-purification of the water might be due to bacterial antagonism.

Recently, much more theoritical approach has been successfully achieved in terms of screening particular organisms with physiological characteristics essential to the production of the specified metabolites. Based on the knowledge accumulated through biochemical and genetical research activities in order to elucidate the mechanisms of antibacterial action of beta-lactam compounds, those mutant strains of Gram-negative organisms which have come to hypersensitive to the specified beta-lactam antibiotic substance have been verified to be a very useful tool for the detection of a novel type of beta-lactam antibiotic substance.

A) Screening Procedures using Beta-lactam-hypersensitive Organisms:

It is now well-known that the mechanisms of antibacterial action of beta-lactam antibiotics depend on the inactivation of the transpeptidase which takes part in the final stage of bacterial cell wall synthesis.

The fact was suggested by the early findings of Fleming that lysis

of staphylococci had taken place in the presence of PC[111]. Then, the chatacteristic of PC action was recognized through the observation that this substance exerted little killing effect except during an active growth phase[112]. Further evidence of an effect of PC on cell wall integrity was demonstrated by the electron micrographs indicating defects in cross wall formation[113].

On the other hand, the detailed process of cell wall synthesis has been elucidated with *Staphylococcus aureus* [114]. However, a cell-free preparation which catalyzed the transpeptidation was found, not in *S. aureus*, the organism in which cell wall synthesis had been studied for many years, but in *Escherichia coli* by the efforts of Japanese biochemists[115].

In *S. aureus*, no carboxypeptidase has been found and peptide-linked oligomers may be as large as decamers according to the above experiment.

There are in fact two terminal reactions in peptidoglycan synthesis in *E. coli*, one of them is the transpeptidation and the other is catalyzed by a D-alanine carboxypeptidase which removes D-alanine residue from the second strand(cf. Fig. 7). Presumably, the action of the carboxypeptidase limits the size of the peptide-linked oligomers in the cell of *E. coli* to dimers.

Both the transpeptidase and the carboxypeptidase are inhibited by PCs and CPNs. The transpeptidase is irreversibly inactivated by PCs, whereas the carboxypeptidase is reversibly inhibited by PCs and activity can be recovered by treatment of inhibited enzyme with PCase. The intact cells of *E. coli* are virtually insensitive to PC G as well as ristocetin, vancomycin and bacitracin which inhibit the preceding steps catalyzed by earlier enzymes. This insensitivity is probably not due to any lack of the sensitive enzyme, but due to the fact that the antibiotics can not penetrate to the site of this enzyme in the bacterial cell.

After then, mutants which are sensitive to antibiotics including PC G have been derived from *E. coli* by various investigators[116].

Genetic investigations have got through on the enzymes concerning cell wall synthesis of Gram-negative bacteria , particularly such as *E. coli* and *Pseudomonas aeruginosa*(116*).

Thus, the presence of PC-binding-proteins(PBP) has been confirmed in the cytoplasmic cell membrane of *E. coli*, and seven different types of PBP components have been detected by isolation of each component[117].

The function as well as the enzymatic activity of these PBP is presented in Table 8 based on the up-to-date information. PBP-1A, 1Bs containing three sub-components, 2 and 3 belong to the category of transpeptidase, whereas, PBP-4, 5 and 6 are the members of carboxy-peptidase.

Mutant strains devoid of PBP-1Bs components have been isolated, and proved to be hypersensitive to almost all beta-lactam antibiotics as presented in Table 9[118]. Although to a lesser extent, those devoid of PBP-5 are also known to show hypersensitivity to the limited number of the antibiotic such as CPNs and PCs(cephamycins and mecillinam are excluded)[119]. The details of the inhibitory effect on PBP components by means of PCs are discussed in the recent review[120].

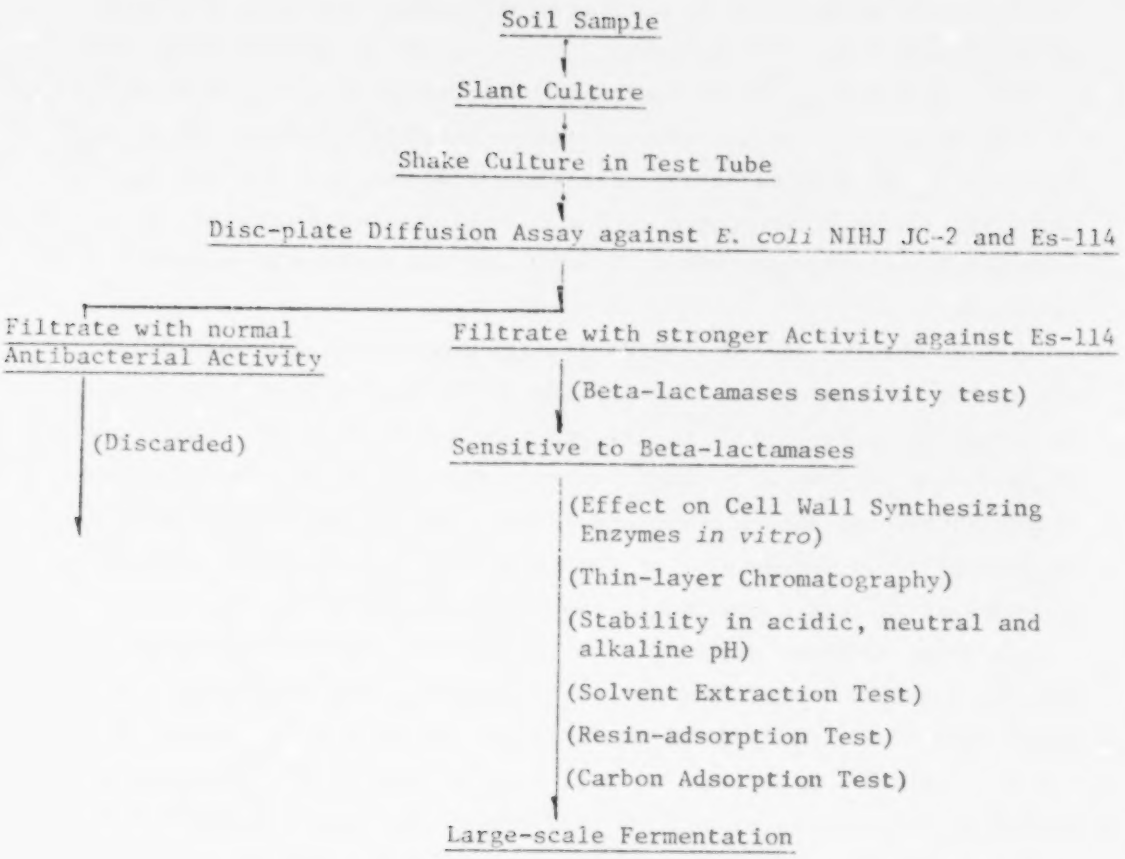
As noted already, usefulness of the beta-lactam-hypersensitive mutants has been exemplified by the discovery of the cephamycin, which might have been predicted by the findings that PC N was produced by a streptomycete[94] as well. In fact, various species of *Streptomyces*, including new species as well as well known spp. like *S. griseus* and *S. halstedii*, were also claimed to produce cephamycins[121].

Many other novel beta-lactam antibiotics have been introduced in terms of the beta-lactam-hypersensitive mutants of Gram-negative bacteria, among which some typical examples may be picked up as follows:

a) Nocardicins by Fujisawa Pharmaceutical Co.[122]

Mutants of *E. coli*(NIHJ JC-2) were obtained after treatment with mutagenic agents and selected for their high sensitivity to PC G. All the mutants acquired hypersensitivity to CPN C, cephamycin C, PC G and Nocardicin A(monocyclic beta-lactam antibiotic). One of the sub-isolates of the mutants was examined for the sensitivity to various antibiotics of natural or semisynthetic origin, and the results are shown in Table 10.

The screening procedures for beta-lactam antibiotics are as illustrated in the following program[123]:


Soil Sample
Slant Culture
Shake Culture in Test Tube
Disc-plate Diffusion Assay against E. coli NIHJ JC-2 and Es-114
Filtrate with normal Antibacterial Activity
(Discarded)
Filtrate with stronger Activity against Es-114
(Beta-lactamases sensivity test)
Sensitive to Beta-lactamases
(Effect on Cell Wall Synthesizing Enzymes in vitro)
(Thin-layer Chromatography)
(Stability in acidic, neutral and alkaline pH)
(Solvent Extraction Test)
(Resin-adsorption Test)
(Carbon Adsorption Test)
Large-scale Fermentation

In the course of above-mentioned screening program, a strain of *Nocardia* was isolated and found to produce a group of antibiotics with interesting properties called Nocardicins. The beta-lactam nucleus, 3-amino-nocardicinic acid, is not obtained naturally, but can be prepared chemically or enzymatically from Nocardicins, and can be used for production of a number of semisynthetic monocyclic beta-lactam antibiotics. See Fig. 8 for chemical structure.

b) Screening of beta-lactam antibiotics using a mutant of *Pseudomonas aeruginosa* by Takeda Chemical Industries Co.[123]

Most strains of *Ps. aeruginosa* are resistant to beta-lactam antibiotics due to so-called intrinsic resistance, in some cases due to

beta-lactamase activity. However, the beta-lactam hypersensitive mutant derived from a beta-lactamase-producing strain of *Pseudomonas aeruginosa* has lost the beta-lactamase function. Through 3-step mutagenesis of parent strain of *Ps. aeruginasa*(Ps) with N-methyl-N'-nitro-N-nitrosoguanidine, a mutant PsC^{ss} was obtained. The sensitivity of the mutant to various naturally occurring beta-lactam antibiotics, such as PCs, CPNs and Nocardicins, known as monocyclic compound, greatly increased, but the sensitivity to other antibiotics did not increase very much. Sensitivity to inhibitors of cell wall synthesis other than beta-lactams, or inhibitors of cell membrane function, protein and nucleic acid biosynthesis showed no or only slight increase.

For the purpose of detecting beta-lactam antibiotics, the following over-layer technique was utilized using four nutrient agar plates:

Plate 1: seeded with parent strain Ps.

Plate 2: seeded with mutant strain PsC^{ss}.

Plate 3: seeded with mutant strain PsC^{ss} with PCase from *Bacillus cereus*.

Plate 4: seeded with mutant strain PsC^{ss} with CPNase from *Enterobacter cloacae*.

Paperdiscs dipped in culture filtrate were placed on these plates, and incubated for 16 hours at 37° C. The fact that antibacterial activity on PsC^{ss} is stronger than that on Ps suggests the presence of beta-lactam antibiotics. Beta-lactam antibiotics can be divided into four groups according to the sensitivity to PCase and CPNase:

Group I: antibiotics like CPN C or cephamycin C, showing no activity on Plate 4.

Group II: antibiotics such as PCs, showing no activities on both Plate 3 and Plate 4.

Group III: as exemplified by 6-APA, showing no activity on Plate 3, but some activity on Plate 4.

Group IV: substances like Clavulanic acid or Nocardicins, showing activity on both Plate 3 and Plate 4.

Group V: non-beta-lactam antibiotics, showing activity on all four plates.

The culture collection consisting of ca. 30,000 strains of yeasts(500), fungi(10,000), bacteria(10,000) and actinomyctes(10,000) was detected for beta-lactam antibiotic production in terms of the screening system mentioned above. 126 strains, 90 from fungi, and 36 from actinomycetes, produced CPN-type antibiotics and PC N, or only PC N, and 25 strains all from fungi produced PC G-type antibiotics. However, strains which produced both CPN antibiotics and PC G-type antibiotics were not detected. The producers of beta-lactam antibiotics have been found in fungi and actinomycetes, but not in yeasts and bacteria[124].

Producers of CPN antibiotics were found in the genera *Arachnomyces*, *Anixiopsis*, as well as in various species of the genus *Emericellopsis* of *Ascomycetes*, and *Spiroidium* and *Paecilomyces* as well as *Cephalosporium* of *Fungi imperfecti*, and *Streptomyces* of *Actinomycetes*. On the other hand, strains of the genera *Thermoascus* and *Gymnoascus* of *Ascomycetes*, and *Polypaecilum* of *Fungi imperfecti* were found to produce PC G-type antibiotics.

Deacetoxy-CPN C, known to be derived chemically from CPN C, was found in the culture broth of all fungal producers such as *Arachnomyces*, *Anixiopsis* and *Spiroidium*[124].

A novel PC, KPN, was found as a product of *Paecilomyces*, and identified as 6-(5-hydroxyvaleramido) penicillanic acid[125]. CPNs and PC N were also produced together with KPN.

Later on, based on this screening method using PsC^{ss} as well as a hypersensitive mutant of *E. coli*, PG8, lacking chromosomal beta-lactamase and PBP-1B component, as test organism, a series of novel monocyclic beta-lactam antibiotics have been discovered by means of the isolation of particular strains of genus *Pseudomonas*. The substances are Sulfazecin produced by *Ps. acidophilia*[126] and Isosulfazecin by *Ps. mesoacidophilia*[127] with a novel nucleus of 3-amino-monobactamic acid(3-AMA).

Selection of these cultures is made on the acidified media at pH 4.5, which is far beyond the optimum for growth of microorganisms.

Based on the discovery of the natural monobactam substances, a series of 3-AMA derivatives have been prepared for the detection of a potent antibiotic. A novel derivative is now found in terms of chemical synthesis based on L-ascorbic acid under cooperation of Hoffmann La Roche as research partner. The substance(cf. Fig. 8) with the trial number of AMA-1080 by Takeda, or Ro17-2301 by Roche is being accepted as comparable with Azthreonam in fundamental biological activities.

c) Azthreonam by Squibb, USA[128]

Soon after the advent of Sulfazecin, similar novel type of monocyclic beta-lactam antibiotics has been reported by Squibb research group using beta-lactam-hypersensitive mutant strains of *Bacillus licheniformis* showing sensitivity down to 0.1 mcg/ml against beta-lactam antibiotics, as test organism. Totally, seven related molecules have been isolated from screening of more than a million of bacteria.[129]

Genera *Chromobacterium*[130], *Agrobacterium*[131] and *Gluconobacter* are found to produce the novel bactams. In view of the simple chemical structure of nucleus compound 3-AMA, chemical synthesis based on threonine has been developed to produce this key compound[132]. Among a lot of chemically modified 3-AMA derivatives Azthreonam(cf; Fig. 8) is picked up because it has shown very strong antibacterial activity against Gram-negative organisms including *Ps. aeruginosa* as well as stable insensitivity to all beta-lactamases except R-plasmids mediating PCase.

B) Screening Procedures for Beta-lactamase Inhibitors:

Resistance of pathogenic organisms against beta-lactam antibiotics, such as PC and CPN is mainly caused by the production of beta-lactamases.

If effective beta-lactamase inhibitor could be obtained, they would show synergy with beta-lactam antibiotics susceptible to the enzymes, against resistant pathogens.

Several research groups have been engaged in the screening of beta-lactamase inhibitors, and a number of compounds with such activity have been reported[133]. Interestingly, all of the inhibitors proved to be beta-lactams of novel structure, therefore, the screening system for such innibitors can be applied for the detection of beta-lactam antibiotics containing anti-beta-lactamase activity.

Principle of the procedure is synergy between beta-lactamase inhibitors and beta-lactamase-sensitive antibiotics against microorganisms producing the enzyme. According to Brown[133], the detection of the inhibitors can be made as follows: nutrient agar plates containing 10 mcg/ml of PC G are seeded with *Klebsiella aerogenes* NCTC418, beta-lactamase producer. Test samples of filtered culture broth are placed in holes cut in the agar plates, and incubated overnight at 37° C. Samples containing a diffusible beta-lactamase inhibitor will give rise to definite zone of inhibition resulting from the protection of the PC contained in the plates. Beta-lactamase inhibitors so far reported are summarized in Fig. 8.

In terms of this detection method, *Streptomyces clavuligerus* was found by Beecham Laboratories, U.K., and the product was named Clavulanic acid. The structure of the substance is a kind of oxapenam compound with beta-lactam ring fused with oxazolidine. Clavulanic acid shows a broad antibiotic spectrum but poor antibacterial activity. However, it was found that various beta-lactamases(but not all) were strongly inhibited by this substance at the concentration less than 10 mcg/ml[134]. A compounded form of the inhibitor together with AMPC was proved to be effective against beta-lactamase-producing pathogens on oral administration, particularly under the composition of potassium clavulanate : AMPC = 125 mg : 250 mg. The complex was named Augmentin in consideration of future marketing.

On the other hand, another different type of beta-lactamase inhibitor has been developed by chemical synthesis by Pfizer Co., USA, and named Sulbactam, since the substance was a penicillanic acid sulfon[135]. The substance acts on some resistant pathogens as well, as beta-lactamase inhibitor.

Synergetic reaction was confirmed against the CPZ-resistant organisms, when a complex of Sulbactam and CPZ(1 : 1) was applied intravenously.

Furthermore, ABPC-methylene-Sulbactam binding in ester form showed a binary function, such as anti-beta-lactamase activity as well as bioavailability in terms of prodrug formation. The resultant compound was named Sultamicillin and developed for a particular prodrug of ABPC with anti-beta-lactamase activity.

Another naturally occurring beta-lactamase inhibitor known as Thienamycin has been developed by Merck, Sharp & Dohme, USA, in terms of the species of *Streptomyces*[136]. In this case, the method of screening is not known, since no information has been given by the discoverers.

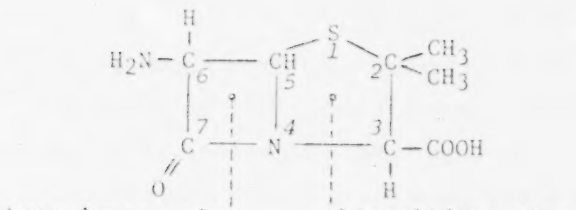
However, a variety of analogous substances now called the carbapenems have been found in Japan in terms of utilizing either hypersensitive mutant or synergetic detection as mentioned before.

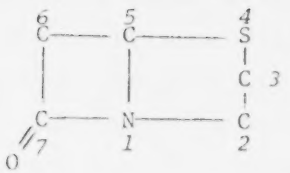
Thienamycin shows strong antibacterial activity against a wide variety of pathogenic organisms as well as considerably sharp insensitivity to beta-lactamases. However, the substance has been knwon to be rather unstable both *in vitro* and *in vivo*, therefore, modified derivatives are essential to assure the chemical stability, in addition, certain inhibitor for dehydropeptidase which derives from swine nephrogeneous enzymes and is knwon to inactivate thienamycin, has to be detected[137].

Imipenem, N-formimidoyl thienamycin, will be the answer for the chemical stability, and cilastatin, an enzyme inhibitor, will give the solution for biological instability. Both substances have been reported by research institute of Merck Co. very recently[138,139].

Further intensive study on the potent derivatives of thienamycin is still under way until the final goal will be attained.

FIGURE 1: BETA-LACTAM RING SYSTEMS

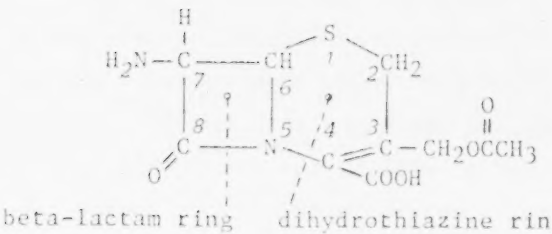


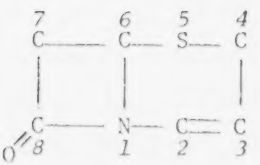


[penam ring] system

1-azabicyclo(3,2,0)-7-oxo-4-thia-heptane

6-AMINOPENICILLANIC ACID





[3-cephem ring] system

1-azabicyclo(4,2,0)-8-oxo-5-thia-oct-2-ene

7-AMINOCEPHALOSPORANIC ACID

FIGURE 1A: PENAM COMPOUNDS (1)

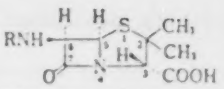
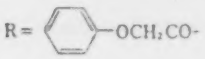
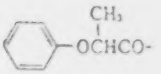
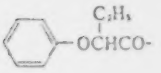
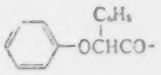
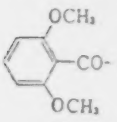
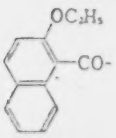
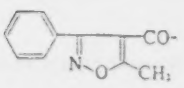
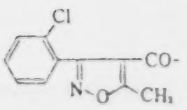
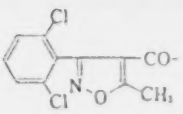
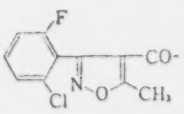
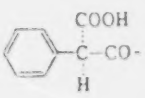
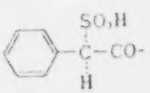
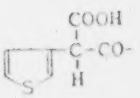
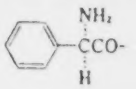
semi-synthetic penicillin(s)	
penicillin V (phenoxymethylpenicillin)	
phenethicillin (α-phenoxyethylpenicillin)	
propicillin (α-phenoxypropylpenicillin)	
phenbencillin (α-phenoxybenzylpenicillin)	
methicillin (2, 6-dimethoxyphenylpenicillin)	
nafcillin (2-ethoxy-1-naphthylpenicillin)	
oxacillin (5-methyl-3-phenyl-4-isoxazolylpenicillin)	
cloxacillin (5-methyl-3-o-chlorophenyl-4-isoxazolylpenicillin)	
dicloxacillin (5-methyl-3-o-dichlorophenyl-4-isoxazolylpenicillin)	
flucloxacillin (5-methyl-3-o-fluorochlorophenyl-4-isoxazolylpenicillin)	
carbenicillin (α-carboxybenzylpenicillin)	
sulbenicillin (α-sulfobenzylpenicillin)	
ticarcillin	

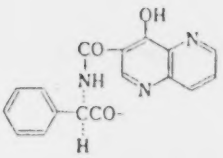
FIGURE 1B: PENAM COMPOUNDS (2)

semi-synthetic penicillin(s)

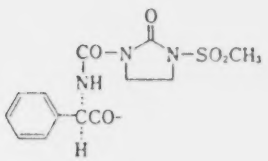
ampicillin (α-aminobenzylpenicillin) R=



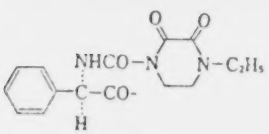
apalcillin



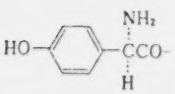
mezlocillin



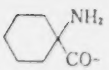
piperacillin



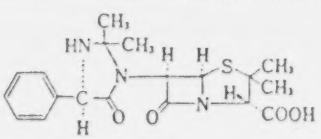
amoxicillin
(D-α-amino-4-hydroxybenzylpenicillin)



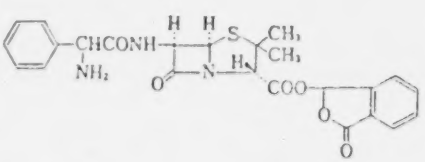
cyclacillin (α-aminocyclohexylpenicillin)



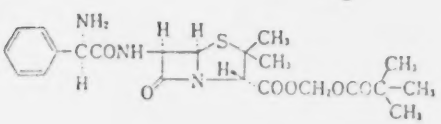
hetacillin (6-(2, 2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-penicillanic acid)



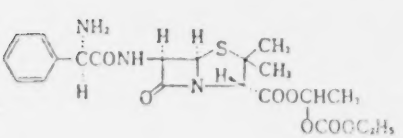
talampicillin



pivampicillin



bacampicillin



mecillinam

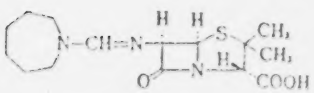


FIGURE 1C: 3-CEPHEM COMPOUNDS (1)

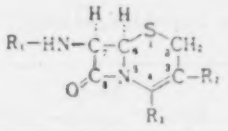
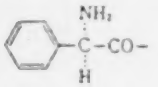
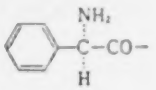
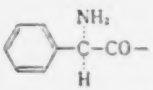
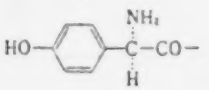
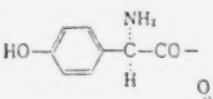
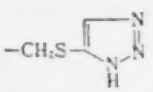
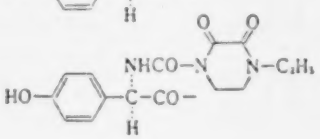
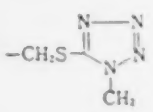
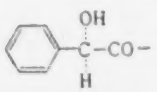
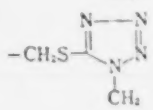
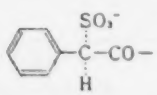
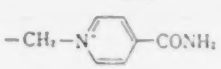
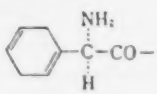
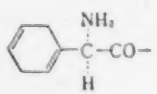
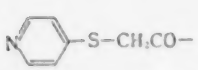
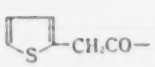
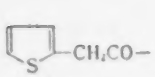
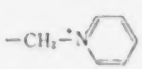
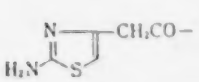
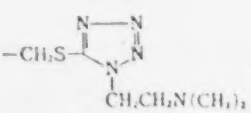
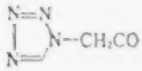
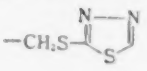
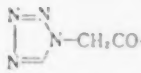
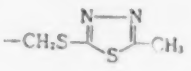
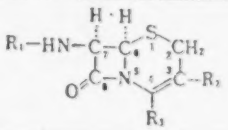
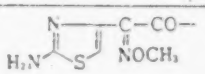
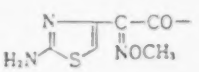
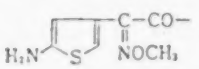
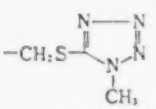
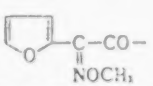
semi-synthetic cephalosporin(s)			
cefalexin (cephalexin)	R_1= 	R_2=—CH_3	R_3=—COOH
cefaclor		—Cl	—COOH
cefaloglycin (cephaloglycin)		—CH_2OCOCH_3	—COOH
cefadroxil		—CH_3	—COOH
cefatrizine			—COOH
cefoperazone			—COOH
cefamandole			—COOH
cefsulodin			—COOH
cefradine		—CH_3	—COOH
cefroxadine		—OCH_3	—COOH
cefapirin (cephapirin)		—CH_2OCOCH_3	—COOH
cefalothin (cephalothin)		—CH_2OCOCH_3	—COOH
cefaloridine (cephaloridine)			—COO^-
cefotiam			—COOH

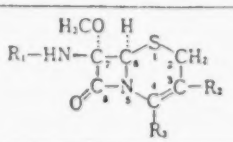
FIGURE 1D: 3-CEPHEM COMPOUNDS (2)

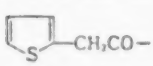
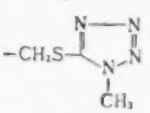
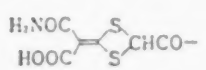
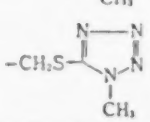
semi-synthetic cephalosporin(s)

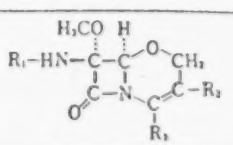
ceftezole	R_1= 	R_2= 	R_3=—COOH
cefazolin			—COOH
cefacetrile (cephacetrile)	$N\equiv C-CH_2CO-$	$-CH_2OCOCH_3$	—COOH

semi-synthetic methoxyiminocephalosporin(s) 

ceftizoxime	R_1= 	R_2=—H	R_3=—COOH
cefotaxime		$-CH_2OCOCH_3$	—COOH
cefmenoxime			—COOH
cefuroxime		$-CH_2OCONH_2$	—COOH

semi-synthetic cephamycin(s) 

cefoxitin	R_1= 	R_2=$-CH_2OCONH_2$	R_3=—COOH
cefmetazole	$N\equiv CCH_2SCH_2CO-$		—COOH
cefotetan			—COOH

oxadethiacephamycin 

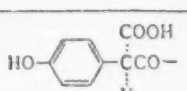
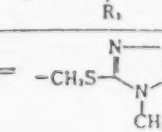
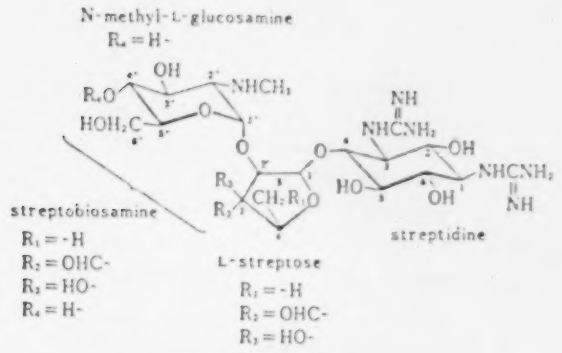
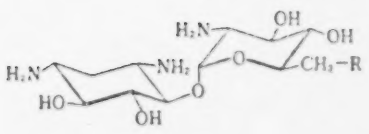
latamoxef (moxalactam)	R_1= 	R_2= 	R_3=—COOH

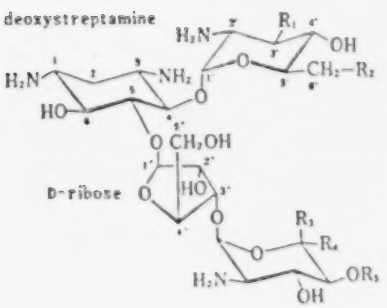
FIGURE 2: CHEMICAL STRUCTURE OF AMINOGLYCOSIDE ANTIBIOTIC (1)



	R_1	R_2	R_3	R_4
streptomycin	—H	OHC—	HO—	H—
dihydrosreptomycin	—H	HOH_2C—	HO—	H—
deoxydihydrostreptomycin	—H	HOH_2C—	H—	H—
hydroxystreptomycin	—OH	OHC—	HO—	H—
mannosidostreptomycin	—H	OHC—	HO—	D-mannose—



reamine (=neomycin A) R = $-NH_2$
paromamine —OH



	R_1	R_2	R_3	R_4	R_5
glucosamine	—OH	—OH			
neosamine C	—OH	$-NH_2$			
neosamine B			$-CH_2NH_2$	—H	—H
neosamine C			—H	$-CH_2NH_2$	—H

	R_1	R_2	R_3	R_4	R_5
neomycin B	—OH	$-NH_2$	$-CH_2NH_2$	—H	—H
neomycin C	—OH	$-NH_2$	—H	$-CH_2NH_2$	—H
paromomycin	—OH	—OH	$-CH_2NH_2$	—H	—H
paromomycin II	—OH	—OH	—H	$-CH_2NH_2$	—H
mannosylparomomycin	—OH	—OH	$-CH_2NH_2$	—H	—D-mannose
lividomycin A	—H	—OH	$-CH_2NH_2$	—H	—D-mannose
lividomycin B	—H	—OH	$-CH_2NH_2$	—H	—H

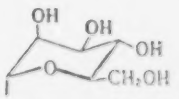
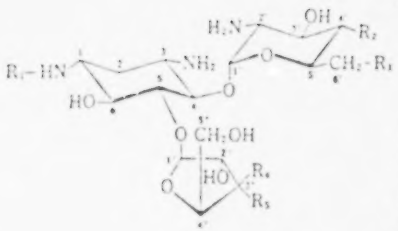
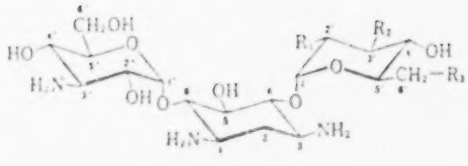


FIGURE 2: CHEMICAL STRUCTURE OF AMINOGLYCOSIDE ANTIBIOTIC (2)

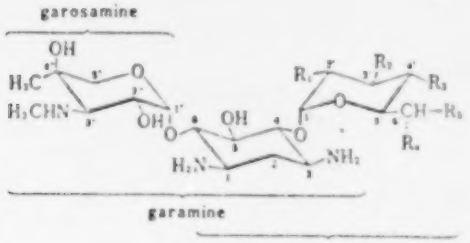


	R_1	R_2	R_3	R_4	R_5
ribostamycin	H—	—OH	$-NH_2$	—H	—OH
xylostatin	H—	—OH	$-NH_2$	—OH	—H
ribosylparomamine (=LL-BM 40S)	H—	—OH	—OH	—H	—OH
butirosin A	$H_2NCH_2CH_2CHCO-$ (OH)	—OH	$-NH_2$	—OH	—H
butirosin B	$H_2NCH_2CH_2CHCO-$ (OH)	—OH	$-NH_2$	—H	—OH
4'-deoxybutirosin A (=Bu-1975 C_1)	$H_2NCH_2CH_2CHCO-$ (OH)	—H	$-NH_2$	—OH	—H
4'-deoxybutirosin B (=Bu-1975 C_2)	$H_2NCH_2CH_2CHCO-$ (OH)	—H	$-NH_2$	—H	—OH
Bu-1709 E_1	$H_2NCH_2CH_2CHCO-$ (OH)	—OH	—OH	—OH	—H
Bu-1709 E_2	$H_2NCH_2CH_2CHCO-$ (OH)	—OH	—OH	—H	—OH



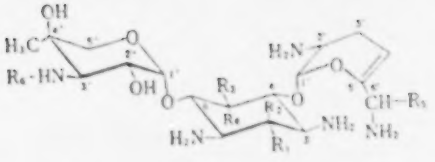
	R_1	R_2	R_3
kanamycin A	HO—	—OH	$-NH_2$
kanamycin B	H_2N—	—OH	$-NH_2$
kanamycin C	H_2N—	—OH	—OH
tobramycin[333]	H_2N—	—H	$-NH_2$

FIGURE 2: CHEMICAL STRUCTURE OF AMINOGLYCOSIDE ANTIBIOTIC (3)



	R_1	R_2	R_3	R_4	R_5
gentamine C_1	H_2N-	—H	—H	$-NHCH_3$	$-CH_3$
gentamine C_{1a}	H_2N-	—H	—H	$-NH_2$	—H
gentamine C_2	H_2N-	—H	—H	$-NH_2$	$-CH_3$

	R_1	R_2	R_3	R_4	R_5
gentamicin B_1	HO—	—OH	—OH	$-NH_2$	$-CH_3$
antibiotic G 418	H_2N-	—OH	—OH	—OH	$-CH_3$
antibiotic JI-20 B	H_2N-	—OH	—OH	$-CH_3$	$-NH_2$
gentamicin C_1	H_2N-	—H	—H	$-NHCH_3$	$-CH_3$
gentamicin C_{1a}	H_2N-	—H	—H	$-NH_2$	—H
gentamicin C_2	H_2N-	—H	—H	$-NH_2$	$-CH_3$
gentamicin C_{2a}	H_2N-	—H	—H	$-CH_3$	$-NH_2$
(gentamicin C_{2a} ≙ C_2 の 6'-epimer)					
gentamicin C_{2b}[138,180] (sagamicin)	H_2N-	—H	—H	$-NHCH_3$	—H



	R_1	R_2	R_3	R_4	R_5	R_6
sisomicin	—H	—H	—OH	—H	—H	H_3C-
mutamicin 1	—OH	—H	—OH	—H	—H	H_3C-
mutamicin 1_a	—OH	—H	—OH	—H	—H	H_3CCO-
mutamicin 1_b	—OH	—H	—OH	—H	—H	H—
mutamicin 2	—H	—H	—H	—H	—H	H_3C-
mutamicin 4	—H	—OH	—OH	—H	—H	H_3C-
mutamicin 5	—H	—H	$-NH_2$	—H	—H	H_3C-
mutamicin 6	—H	—H	—H	—OH	—H	H_3C-
verdamicin	—H	—H	—OH	—H	$-CH_3$	H_3C-

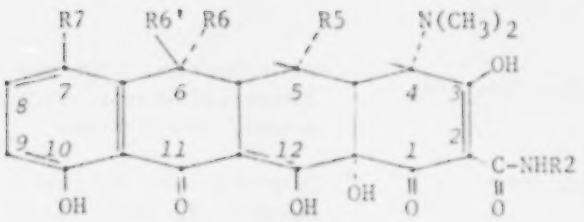
Fig. 3: RELATIVE ANTIBACTERIAL ACTIVITY OF CEPHEM ANTIBIOTICS

	(0.02-0.1)	(0.1 - 1)	(1 - 10)	(10 - 100)	(>100)
S. aureus		CER	CET/CTZ/CEZ/CMZ/CTM/cmx/CXM/cpz/CFX	czx/lmox	
S. epidermidis		CER/CET/CTZ/	CEZ/CTM/cpz/CXM/cmx/czx/CMZ=ctx/CFX	lmox	
H. influenza	ctx/cpz/cmx=lmox	CXM	CMZ	CEZ	
E. coli		ctx/czx=cmx/lmox/CTM/cpz	CMZ=CEZ=CTZ/CXM/CFX/CER	CET	
K. pneumoniae	czx	ctx/cmx/lmox/CTM/cpz	CMZ/CXM=CEZ/CFX/CER=CET		
C. freundii		ctx/lmox	cmx/czx/cpz/CTM	CXM/CMZ/CTZ/CEZ/CET/CER	CFX
Ent. cloacae		czx	cmx=lmox/ctx/cpz/CTM	CXM/CER/CMZ	CEZ/CET/CFX/CTZ
Ent. aerogenes		cmx/czx/ctx/imox	cpz/CTM	CXM/CEZ=CTZ	CET=CFX/CER=CMZ
Ser. marcescens			cmx/czx	ctx/lmox/cpz	CEZ=CET=CTZ=CER/CTM=CXM=CMZ=CFX
Pro. mirabilis		czx/cmx=ctx/lmox/CTM	cpz/CXM/CMZ/CEZ=CTZ/CFX/CET	CER	
Pro. vulgaris		czx/cmx/lmox/ctx	cpz/CFX	CMZ/CTM	CEZ=CER=CTZ/CET=CXM
Pro. morganii		lmox/cmx/ctx	czx/cpz/CMZ	CTM/CFX/CXM	CEZ=CER=CTZ/CET
Ps. aeruginosa			cfs	cpz/cmx/ctx=lmox/czx	CEZ=CMZ/CXM
B. fragilis			lmox/ctx/CFX	cmx=CMZ/czx/cpz=CEZ=CTM	

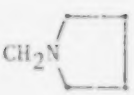
Remarks: *S: Staphlococcus, H: Haemophilus, E: Escherichia, K: Klebsiella, C: Citrobacter, Ent: Enterobacter, Ser: Serratia, Pro: Proteus, Ps: Pseudomonas, B: Bacteroides*

MIC is given as mcg/ml. Small letter stands for 3rd generation, capital letter does 1st generation, underlined 2nd generation.

FIGURE 4: NAPHTHACENIC ANTIBIOTICS, TETRACYCLINES & ANTHRACYCLINES



(Tetracyclines)

(Generic Name)	(R2)	(R5)	(R6)	(R6')	(R7)
CTC	H	H	OH	CH_3	Cl
OTC	H	OH	OH	CH_3	H
TC	H	H	OH	CH_3	H
DMTC	H	H	OH	H	Cl
Roli-TC		H	OH	CH_3	H
Methacycline	H	OH	CH_2		H
Doxycycline	H	OH	H	CH_3	H
Minocycline	H	H	H	H	$N(CH_3)_2$

(Chromophore group of Anthracyclines)

FIGURE 5: 7-ACYLAMINO GROUPS TAKEN UP BY BACTERIAL ACYLASES

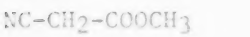
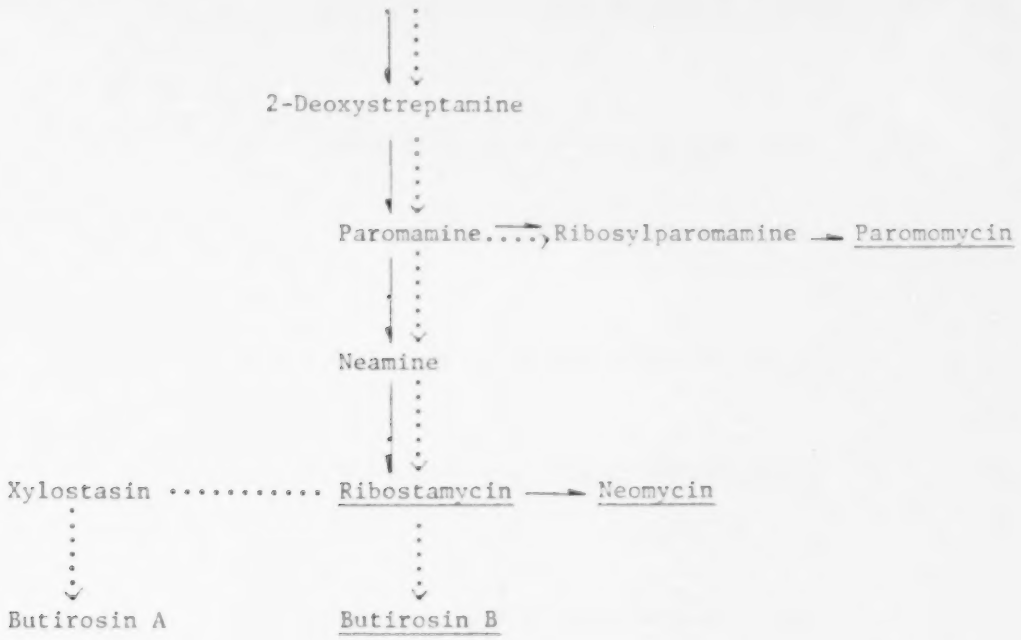
[Side-chain Acids, in ester form]	[Cephem Compound]	[Organism]
	CEX, CEG, CCL	*Acetobacter, Achromobacter, Kluyvera, Xanthomonas, Pseudomonas*
	CFD, CFT	*Acetobacter, Achromobacter, E. coli, Azotobacter, Pseudomonas, Thiobacillus*
	CED, CXD	*Achromobacter, Azotobacter*
	CET, CER	*Bacillus megaterium*
	CEZ	*Kluyvera, Arthrobacter, Bacillus megaterium*
	CEC	*Bacillus megaterium*
	CEM	*Bacillus megaterium*

FIGURE 6: PROBABLE BIOSYNTHETIC PATHWAYS OF AMINOGLYCOSIDE ANTIBIOTICS



Remarks: ..... by *Bacillus circulans*

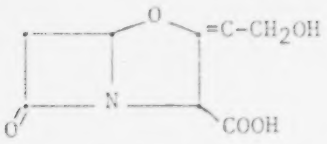
———— by *Streptomyces sp.*

FIGURE 7: REACTIONS CATALYZED BY PEPTIDOGLYCAN TRANSPEPTIDASE AND D-ALANINE CARBOXYPEPTIDASE IN E. coli

Remarks: GlcNAc stands for N-acetylglucosamine;
MurNAc stands for N-acetylmuramic acid;
meso-DAP stands for meso-diaminopimelic acid.

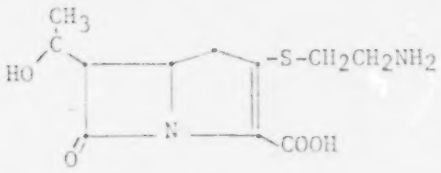
FIGURE 8: BETA-LACTAMASE INHIBITORS & MONOCYCLIC AZETIDINONE ANTIBIOTICS

(A) Penam Nucleus:

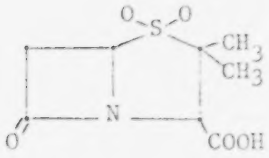


(Clavulanic Acid)

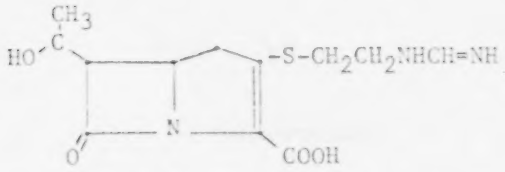
(B) Penem Nucleus:



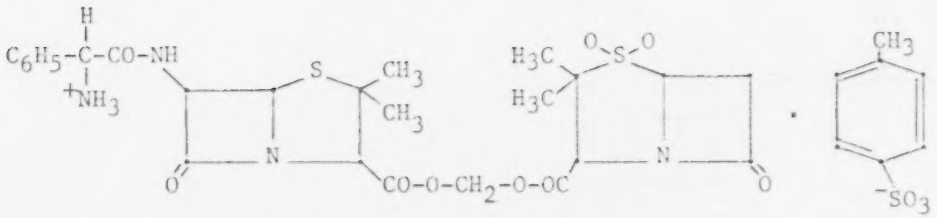
(Thienamycin)



(Sulbactam)

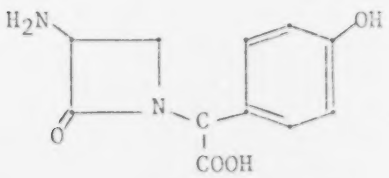


(Imipenem)

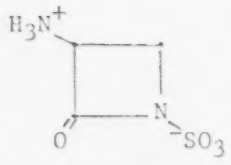


(Sultamicillin)

(C) Azetidinone Nucleus:



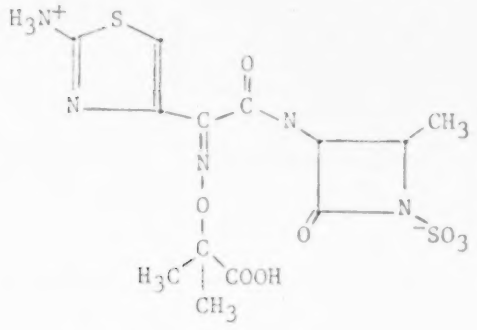
(3-amino-nocardicinic Acid)



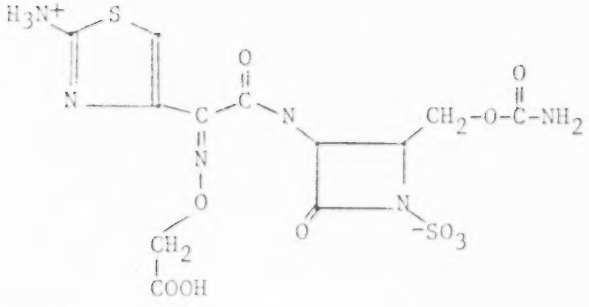
(3-amino-monobactam Acid)

FIGURE 8: BETA-LACTAMASE INHIBITORS & MONOCYCLIC AZETIDINONE ANTIBIOTICS

(D) Monobactam Antibiotics:



(Azthreonam)



(AMA-1080)

TABLE 1: MANUFACTURERS FOR LISTED ANTIBIOTIC DRUGS IN 1982

	(Name)	(Address)	(Phone)
1	Banyu Seiyaku K.K.:	2-7 Honcho, Nihonbashi, Chuo-ku, Tokio	(03-270-7551)
2	Beecham Yakuhin K.K.:	c/o Shinanpeidai-Tokyu Bldg. 1-21-2 Dogenzaka, Shibuya-ku, Tokio	(03-403-3211)
3	Bristol-Banyu K.K.:	Nihonseimei-Akasaka Daini Bldg., 7-1-16 Akasaka, Minato-ku, Tokio	(03-403-3211)
4	Bristol-Myers K.K.:	Ibid.	(Ibid.)
5.	Chugai Seiyaku K.K.:	c/o Kyobashi-Chiyoda Bldg., 2-1-9 Kyobashi, Chuo-ku, Tokio	(03-281-6611)
6	Daiichi Seiyaku K.K.:	3-14-10 Nihonbashi, Chuo-ku, Tokio	(03-272-0611)
7	Dainippon Seiyaku K.K.:	3-25 Dosho-machi, Higashi-ku, Osaka	(06-203-5321)
8	Dojin Iyakukako K.K.:	5-2-2 Yayoi-cho, Nakano-ku, Tokio	(03-382-3773)
9	S. S. Seiyaku K.K.:	2-12-4 Hama-cho, Nihonbashi, Chuo-ku, Tokio	(03-668-4511)
10	Essex Japan K.K.:	4-1-20 Itachibori, Nishi-ku, Osaka	(06-532-3232)
11	Fuji Seiyaku Kogyo K.K.:	1-9-11 Shikahama, Adachi-ku, Tokio	(03-899-5003)
12	Fujimoto Seiyaku K.K.:	1-3-40 Nishiotsuka Matsubara-shi, Osaka	(0723-32-5151)
13	Fujisawa Yakuhin Kogyo K.K.:	4-3 Dosho-machi, Higashi-ku, Osaka	(06-202-1141)
14	Hishiyama Seiyaku K.K.:	2-37 Dosho-machi, Higashi-ku, Osaka	(06-231-9845)
15	Hoechst Japan K.K.:	8-10-16 Akasaka, Minato-ku, Tokio	(03-479-5111)
16	Hokuriku Seiyaku K.K.:	1-3-14 Tachikawa-cho, Katsuyama-shi, Fukui	(07798-8-5111)
17	K.K. Isei:	2-9-19 Kasumi-cho, Yamagata-shi, Yamagata	(0236-22-7755)
18	Itoyoshi Seiyaku G.K.:	3-6 Dosho-machi, Higashi-ku, Osaka	(06-231-o288)
19	Kaken Seiyaku K.K.:	2-28-8 Honkomagome, Bunkyo-ku, Tokio	(03-946-2111)
20	Kanebo K.K.:	1-3-12 Motoakasaka, Minato-ku, Tokio	(03-478-4441)
21	Kantoishi Seiyaku K.K.:	c/o Shijuku-Mitsui Bldg., 2-1-1 Nishishinjuku, Shijuku-ku, Tokio	(03-344-5411)
22	Kissei Yakuhin Kogyo K.K.:	19-48 Yoshino Matsumoto-shi, Nagano	(0263-25-9081)
23	Kotobuki Seiyaku K.K.:	6351 Sakashiro, Sakashiro-cho, Hanishina-gun, Nagano	(02688-2-2211)
24	Kyorin Seiyaku K.K.:	2-5 Kandasurugadai, Chiyoda-ku, Tokio	(03-293-3411)
25	Kyowa Hakko Kogyo K.K.:	c/o Otemachi Bldg., 1-6-1 Ote-machi, Chiyoda-ku, Tokio	(03-201-7211)

26 Maruko Seiyaku K.K.: 1-5-17 Kodama, Nishi-ku, Nagoya(052-524-2371)

27 Meiji Seika K.K.: 2-4-16 Kyobashi, Chuo-ku, Tokio(03-272-0500)

28 K.K. Midorijuji: 1-15-1 Imabashi, Higashi-ku, Osaka(06-228-0700)

29 Mochida Seiyaku K.K.: 1-7 Yotsuya, Shinjuku-ku, Tokio(03-358-7211)

30 K.K. Mohanyakuhin Kenkyujo: 3-39-12 Sengoku, Bunkyo-ku, Tokio(03-917-9281)

31 Morishita Seiyaku K.K.: 4-29 Dosho-machi, Higashi-ku, Osaka(06-203-5512)

32 Nihon Kemifa K.K.: 2-2-3 Iwamoto-cho, Chiyoda-ku, Tokio(03-863-1211)

33 Nihon Chiba-Geigy K.K.: 10-66 Miyuki-cho, Takarazuka-shi, Hyogo(0797-71-1171)

34 Nihon Glaxo K.K.: c/o Shinjuku-Sumitomo Bldg., 2-6-1 Nishishinjuku, Shinjuku-ku, Tokio(03-344-6611)

35 Nihon Iyakuhin Kogyo K.K.: 1-6-21 Sokuruwa, Toyama-shi, Toyama(0764-32-2121)

36 Nihon Kayaku K.K.: 1-2-1 Marunouchi, Chiyoda-ku. Tokio(03-264-1266)

37 Nihon Lederle K.K.: 1-10-3 Kyobashi, Chuo-ku, Tokio(03-561-8781)

38 Nihon Merck-Banyu K.K.: 3-9-2 Nihonbashi, Chuo-ku, Tokio(03-271-6241)

39 Nihon Roussel K.K: 4-5 Muro-machi, Nihonbashi, Chuo-ku, Tokio(03-241-7731)

40 Nihon Shoji K.K.: 2-30 Koku-cho, Higashi-ku, Osaka(06-941-0301)

41 Nihon Squibb K.K.: c/o Nagai-Int'l Bldg., 2-12-19 Shibuya, Shibuya-ku, Tokio (03-400-8441)

42 Nihon Upjohn K.K.: c/o Shinjuku-Sumitomo Bldg., 2-6-1 Nishishinjuku, Shinjuku-ku, Tokio(03-347-8500)

43 Nikken Kagaku K.K.: 5-4-14 Tsukiji, Chuo-ku, Tokio(03-544-8701)

44 Otsuka Seiyaku K.K.: 2-9 Tsukasa-cho, Kanda, Chiyoda-ku, Tokio(03-292-0021)

45 Sanko Seiyaku Kogyo K.K.: 4-2 Hongoku-cho, Nihonbashi, Chuo-ku, Tokio(03-279-3911)

46 Sankyo K.K.: 2-7-12 Ginza, Chuo-ku, Tokio(03-562-0411)

47 Sanraku-Ocean K.K.: 1-15-1 Kyobashi, Chuo-ku, Tokio(03-562-1211)

48 Santen Seiyaku K.K.: 3-9-19 Shimoshinjo, Yodogawa-ku, Osaka(06-328-2666)

49 K.K. Sanwa Kagaku Kenkyujo: 35 Higashisotobori-cho, Higashi-ku, Nagoya(052-951-8130)

50 Sato Yakuhin Kogyo K.K.: 9-2 Kanonji-cho, Kashiwara-shi, Nara(07442-8-0021)

51 Sawai Seiyaku K.K.: 1-4-25 Akagawa, Asahi-ku, Osaka(06-928-7071)

52 Shin-Nihon Jitsugyo K.K.: 3-2-9 Ginza, Chuo-ku, Tokio(03-561-3181)

53 Shionogi Seiyaku K.K.: 3-12 Dosho-machi, Higashi-ku, Osaka(06-202-2161)

54 Sumitomo Kagaku Kogyo K.K.: 5-15 Kitahama, Higashi-ku, Osaka(06-202-0051)

55 Showa Shinyaku K.K.: 1-2-7 Imaike Chigusa-ku, Nagoya(052-741-4311)

56 Showa Yakuhin Kako K.K.: 1-16-5 Kyobashi, Chuo-ku, Tokio(03-567-9571)

57 Taito-Pfizer K.K.: P.O.Box 226, Shinjuku-ku, Tokio(03-344-4411)

58 Taiyo Yakuhin Kogyo K.K.: 2-181 Nishimoto-Issiki-cho, Takayama-shi, Gifu (0577-33-0811)

59 Takada Seiyaku K.K.: 203-1 Miyamae-cho, Omiya-shi, Saitama(0486-22-2626)

60 Takeda Yakuhin Kogyo K.K.: 2-27 Dosho-machi, Higashi-ku, Osaka(06-204-2111)

61 Tatsumi Kagaku K.K.: 3-345 Mitsuuma, Kanazawa, Ishikawa(0762-47-2131)

62 Teisan Seiyaku K.K.: 2-9 Honcho, Nihonbashi, Chuo-ku, Tokio(03-242-2311)

63 Tobishi Yakuhin Kogyo K.K.: c/o Yurakucho Bldg., 1-10-1 Yuraku-cho, Chiyoda-ku, Tokio(03-213-3771)

64 Toho Yakuhin Kogyo K.K.: c/o Yachiyo Bldg., 1-14 Awaji-cho, Higashi-ku, Osaka (06-201-4881)

65 Torii Yakuhin Kogyo K.K.: 3-3-14 Honcho, Nihonbashi, Chuo-ku, Tokio(03-241-3101)

66 Toyama Kagaku Kogyo K.K.: 3-2-5 Nishishinjuku, Shinjuku-ku, Tokio(03-348-6611)

67 Towa Yakuhin K.K.: 1085-1, Sanban, Kadoma-shi, Osaka(06-908-2195)

68 Toyo Jozo K.K.: 4-5-13 Shibaura, Minato-ku, Tokio(03-454-7511)

69 Toyo Shinyaku K.K.: 1-22 Asahicho, Nishibiwajima-cho, Kasugai-gun, Aichi(052-503-0131)

70 Tsuruhara Seiyaku K.K.: 1-16-1 Toyoshimakita, Ikeda-shi, Osaka(0727-61-1456)

71 Yamanouchi Seiyaku K.K.: 2-5 Honcho, Nihonbashi, Chuo-ku, Tokio(03-244-3000)

72 Yoshitomi Seiyaku K.K.: 3-35 Hirano-cho, Higashi-ku, Osaka(06-201-1161)

73 Nihon Wyeth K.K.: 4-15-21 Nishiazabu, Minato-ku, Tokio(03-407-6981)

TABLE 2: HISTORICAL REVIEW ON ANTIBIOTICS MARKETED IN JAPAN

(Date)	(Name of Substance)	(Characteristics)	(Origin)
1950	Chloramphenicol	Wide Spectrum	Parke Davis
50	Chlortetracycline	Ibid.	Lederle
50	Oxytetracycline	Ibid.	Pfizer
51	Streptomycin	Anti-TB	
51	Colistin	Gram(-)/Polypeptide	Lion Kinyaku
53	Erythromycin	Macrolide	Eli Lilly
53	Bacitracin	Gram(+)/Polypeptide	
53	Fradiomycin	GRam(-)/Aminoglycoside	
54	Tetracycline	Wide Spectrum	Lederle
54	Sarkomycin	Oncostatic	Meiji Seika
54	Viomycin	Anti-TB	Pfizer
56	Penicillin V	Oral PC(Penam)	
56	Carbomycin	Macrolide	Pfizer
56	Kitasamycin	Ibid.	Toyo Jozo
57	Cycloserine	Anti-TB	Eli Lilly
57	Novobiocin	Gram(+)	Upjohn
57	Nystatin	Antifungal	Squibb
57	Oleandomycin	Macrolide	Pfizer
58	Kanamycin	Anti-TB, Gram(+)/(-)	Meiji Seika
59	Phenethicillin	Synthetic Penam/Oral Form	Bristol
59	Rolitetracycline	Modified TC	Hoechst
59	Mitomycin	Oncostatic	Kyowa Hakko
61	Chromomycin A3	Ibid.	Takeda
61	Demethyl TC	Modified TC	Lederle
61	Methicillin	Staphylococcal β-lactamase Resistant	Beecham
62	Oxacillin	Ibid.	Bristol
62	Amphotericin B	Antifungal	Squibb
63	Spiramycin	Macrolide	Rhone Pulenc
63	Ampicillin	Gram(-)/Penam	Beecham
63	Propicillin	Oral Form/Penam	Ibid.
64	Cloxacillin	Staphylococcal β-lactamase Resistant	Bristol

1965	Methacycline	Modified OTC	Pfizer
65	Cephalothin	Cephem/Injectable Form	Eli Lilly
65	Capreomycin	Anti-TB	Ibid.
65	Cephaloridine	Cephem	Glaxo
65	Lincomycin	Gram(+)	Upjohn
67	Dicloxacillin	Penam/Staph. β-lactamase Resistant	Bristol
68	Bleomycin	Oncostatic	Nihon Kayaku
68	Parcmomycin	Aminoglycoside	Farm Italia
68	Gentamicin	Ibid.	Schoering
69	Aminodeoxy-KM	Modified Aminoglycoside	Meiji Seika
69	Carbenicillin	Against Pseudomonas/Penam	Beecham
69	Hetacillin	Modified Ampicillin/Penam	Bristol
69	Doxycycline	Modified OTC	Pfizer
69	Cephaloglycin	Oral Form/Cephem	Eli Lilly
69	Josamycin	Macrolide	Yamanouchi
69	Enramycin	Gram(+)/Peptide	Takeda
70	Daunorubicin	Oncostatic/Anthracycline	Farm Italia
70	Cephalexin	Oral Form/Cephem	Eli Lilly
71	Clindamycin	Modified Lincomycin	Upjohn
71	Minocycline	Modified TC	Lederle
71	Siccanin	Antifungal	Sankyo
71	Cefazolin	Cephem	Fujisawa
71	Rifampicin	Anti-TB	Repeti
71	Cyclacillin	Oral Form/Penam	Wyeth
72	Fusidic Acid	Gram(+)/Steroid Compound	Sankyo
72	Ribostamycin	Aminoglycoside	Meiji Seika
72	Sulbenicillin	Against Pseudomonas/Penam	Takeda
73	Midekamycin	Macrolide	Meiji Seika
74	Dibekacin	Against Pseudomonas/KM Family	Ibid.
74	Doxorubicin	Oncostatic/Anthracycline	Farm Italia
74	Amoxicillin	Oral Form/Penam	Beecham
75	Maridomycin	Macrolide	Takeda
75	Enviomycin	Anti-TB	Toyo Jozo
75	Carindacillin	Oral Form/Modified Carbenicillin	Pfizer

1976	Neocarcinostatin	Oncostatic/Peptide	Kayakukosei
76	Tobramycin	Aminoglycoside	Eli Lilly
76	Amicacin	Modified KM/Aminoglycoside	Bristol-Banyu
76	Minocycline	Modified TC	Lederle
76	Cefapirin	Cephem	Bristol
77	Carfecillin	Oral Form/Carbecillin Family	Beecham
77	Talampicillin	Ampicillin Ester/Penam	Yamanouchi
77	Cefradine	Cephem	Squibb
77	Ceftezole	Ibid./Modified Cephazolin	Fujisawa
77	Cefacetrile	Cephem	Ciba-Geigy
78	Spectinomycin	Aminoglycoside	Upjohn
78	Pivmecillinam	Specific Penam	Leo
79	Piperacillin	Against Pseudomonas/Ampicillin-F.	Toyama Kagaku
79	Ticarcillin	Ibid./Carbenicillin-Family	Beecham
79	Cefmetazole	Cephem/Cephamycin-Family, II	Sankyo
79	Cefoxitin	Ibid./Ibid.	Merck Sharp & D.
79	Cefatrizine	Cephem/Oral Form	Bristol-S.K.F.
80	Fosfomycin	Against Pseudomonas	CEPA-Merck
80	Peplomycin	Oncostatic/Modified Bleomycin	Nihon Kayaku
80	Cefsu'odin	Against Pseudomonas/Cephem,III	Takeda
80	Cefotiam	Cephem, II	Ibid.
81	Vancomycin	Gram(+)/Oral	Eli Lilly
81	Aclarubicin	Oncostatic/Anthracycline	Sanraku-Ocean
81	Bacampicillin	Ampicillin Ester	Atlas
81	Sisomicin	Aminoglycoside	Schoering
81	Cefroxadine	Oral/Modified Cephalexin	Ciba-Geigy
81	Micronomicin	Gentamycin-Family	Kyowa Hakko
81	Cefuroxime	Cephem II	Glaxo
81	Cefoperazone	Cephem III	Toyama Kagaku
81	Cefotaxime	Ibid.	Roussell-Hoechst
81	Latamoxef	Oxacephem/Cephem III	Shionogi
81	Mezlocillin	Ampicillin-Family	Bayer
81	Ceftizoxime	Cephem III	Fujisawa
81	Cefaclor	Oral Form/Cephem II*	Eli Lilly

1982	Cefadroxil	Oral Form/Cephem	Bristol
82	Cefmenoxime	Cephem III	Takeda
83	Cefamandole	Cephem II	Eli Lilly
83	Cefotetan	Cephem III/Cephamycin Series	Yamanouchi
1970*	Flucloxacillin	Penam/Penicillinase Resistant	Beecham

Remarks:
1) Japanese origins are marked with the underline.
2) Cephem II, and III are the presentation of "Generation".
3) The date is signified by the time of Government Approval.
4) Cefaclor may be indicated as a member of 2nd. generation series by some authority, but generally it is not accepted in Japan.

TABLE 3: ANTIBIOTIC DRUGS LISTED IN SOCIAL SECURITY ORGANIZATION

(A) ORAL FORM:

(Code)	(Antibiotic Action)	(Generic Name)
611	Gram-positives	Penicillin V-K/PC G-K/Benzathine PC G Oxacillin-Na/Cloxacillin-Na/Dicloxacillin-Na/ Flucloxacillin-Na/Phenethicillin-K/Propi- cillin-K Lincomycin-HCl/Clindamycin-HCl/Clinda- mycin Palmitate-HCl Fusidin-Na Bancomycin-HCl
612	Gram-negatives	Colistin-H_2SO_4/Colistin Metasulfonate-Na/ Polymixin-B-H_2SO_4 Fradiomycin-H_2SO_4 Paromomycin-H_2SO_4
613	Gram-positives as well as Gram-negatives	Amoxicillin/Ampicillin/Talampicillin/Baca- mpicillin/Pivmecillinam/Calindacillin/Cal- phecillin/Cyclacillin/Hetacillin-K Cephalexin/Cephaloglycin/Cefaclor/Cefa- trizine/Cefadroxil/Cefradine Fosfomycin-Ca
614	Gram-positives/R/V (R: Rickettsia, V: Virus)	Erythromycin/EM Stearate/EM Estrate/EM Ethylsuccinate/Kitasamycin/Acetyl KTM/ Acetyl Spiramycin/Josamycin/JM Propionate/ Oleandomycin Phosphate/Triacetyl OLM/ Midecamycin
615	Gram-positives, Gram- negatives/R/V	Chloramphenicol/CP Palmitate Oxytetracycline-HCl/OTC/Tetracycline/TC- HCl/TC Metaphosphate/Demethyl TC/Demethyl Chlortetracycline/Doxycycline-HCl/Metha- cycline-HCl/Minocycline-HCl

616	Mycobacteria	Cycloserine/Rifampicin/Kanamycin-H_2SO_4
617	Fungi	Nystatin/Griseofluvin/Amphotericin B
618	Cancer	Mitomycin-C
619	(Complex Form)	Ampicillin-Cloxacillin/Ampicillin-Dicloxacillin/TC-OLM/Benzathine PC V-PC V
(B)	INJECTABLE FORM:	
611	Gram-positives	PC G-K/Procain PC G/Benzathine PC G Oxacillin-Na/Cloxacillin-Na/Methicillin-Na Lincomycin-HCl/Clindamycin Phosphate
612	Gram-negatives	Colistin Methanesulfonate-Na/Polymixin-B-H_2SO_4 Gentamicin-H_2SO_4/Micronomicin-H_2SO_4 Dibekacin Ribostamycin-H_2SO_4 Spectinomycin-HCl/Tobramycin/Amikacin-H_2SO_4/Sisomicin-H_2SO_4/Paromomycin-H_2SO_4/Bekanamycin-H_2SO_4
613	Gram-positives as well as Gram-negatives	Ampicillin/AMPC-Na/Carbenicillin-Na/Sulbenicillin-Na/Ticarcillin-Na/Piperacillin-Na/Hetacillin-K/Mezlocillin-Na Cefazolin-Na/Cefacetril-Na/Cefapirin-Na/Cephalothin-Na/Cephaloridine/Cefotiam-HCl/Cefoxitin-Na/Cefotaxime-Na/Cefoperazone-Na/Cefsulodin-Na/Ceftizoxime-Na/Ceftezole-Na/Cefmetazole-Na/Cefuroxime-Na/Cefmenoxime-HCl Fosfomycin-Na
614	Gram-positives/R/V	EM Lactobionate/KTM Tartrate/OLM Phosphate
615	Gram-positives, Gram-negatives/R/V	CP/CP Succinate-Na OTC/Rolitetracycline/RolITC-HNO_3/Doxycycline-HCl/Minocycline-HCl

616	Mycobacteria	Streptomycin-H_2SO_4/Kanamycin-H_2SO_4/Capreomycin-H_2SO_4/Enviomycin-H_2SO_4
617	Fungi	Amphotericin B
618	Cancer	Actinomycin D/Acralubicin-HCl/Daunolubicin-HCl/Doxolubicin-HCl/Bleomycin-HCl/Bleomycin-H_2SO_4/Cromomycin/Mitomycin-C/Neocartinostatin/Peplomycin-H_2SO_4
619	(Complex Form)	AMPC-Oxacillin/AMPC-Cloxacillin/TC-HCl-OLM-Phosphate/Kanamycin-H_2SO_4-Procaine PC G

TABLE 4: MAIN JOURNALS OF ENGLISH EDITION CONCERNING THE TOPICS OF NOVEL ANTIBIOTICS AND ITS RELATED TECHNOLOGY, PUBLISHED IN JAPAN

1) Journal of Antibiotics, published monthly.
Nihon Koseibussitsu Gakujitsu Kyogikai: 2-20-8, Kamiosaki, Shinagawa-ku, Tokio 141(Tel; 03-491-0181)

2) Chemical & Pharmaceutical Bulletin, published monthly.
Nihon Yakugakukai: 2-12-15-501 Shibuya, Shibuya-ku, Tokio 150(Tel; 03-406-3321)

3) Agricultural & Biological Chemistry, published monthly.
Nihon Nogeikagakukai: c/o Gakkai-Center Budg. 2-4-6, Yayoi, Bunkio-ku, Tokio 113(Tel; 03-811-8789)

4) Chemistry Letter, published monthly
Nihon Kagakukai: 1-5, Kanda-Surugadai, Chiyoda-ku, Tokio 101(Tel; 03-292-6161)

5) Fermentation Technology, published bimonthly.
Nihon Hakko Kogakukai: c/o Osaka University, Yamadakami, Suita-shi, Osaka 565(Tel; 06-877-5111)

6) Japan Drug Industry Review, published yearly.
Yakugyo Jiho K. K.: 2-36, Kanda-Jinbo-cho, Chiyoda-ku, Tokio 101(Tel; 03-265-7751)

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TABLE 6: SUMMARY OF BETA-LACTAMASES CLASSIFIED BY RICHMOND

[Bacteria]	[Genes]	[Type by Richmond]	[Function]	[Substrate]	[Producing Organism]
Gram (+)	Plasmids	-	PCase (Inducible)	PC G, PC V, ABPC, AMPC	*Staphylococcus aureus, S. epidermidis; Bacillus cereus*
Gram (-)	Chromosome	I	CPNase	CER, CET, CEX, CEZ, CEG	*Enterobacter, Serratia, Citrobacter, Proteus vulgaris, Pseudomonas aeruginosa*
	Chromosome	II	PCase	ABPC, AMPC, CER	*Proteus morganii, Proteus mirabilis*
	Plasmids	III (TEM)	PCase	ABPC, AMPC, CER	R-factor carrying organisms
	Chromosome	IV	PCase	ABPC, AMPC, CER	*Enterobacter, Klebsiella*
	Plasmids	V (Oxacillinase)	PCase	ABPC, AMPC, MPIPC	Certain type of R-factor carrying organisms, *Pseudomonas aeruginosa*

TABLE 7: PRESENCE OF AMINOGLYCOSIDE-INACTIVATING ENZYMES IN CLINICAL ISOLATES

[Enzyme]	[Substrate]	[Gram +]	[Gram -]	[Producing Strain]
N-acetyltransferases				
AAC (3)	GM, TOB	+	?	+
AAC (2')	GM	+	-	+
AAC (6')	KM	+	+	+
O-nucleotidyltransferases				
AAD (6)	SM	-	+	-
AAD (4')	TOB	-	+	-
AAD (2")	GM	+	?	-
AAD (3", 9)	SM, SPC*	+	+	-
O-phosphotransferases				
APH (6)	SM	+	-	+
APH (3')	KM	+	+	+
APH (2")	GM, TOB	-	+	-
APH (3")	SM	+	+	+
APH (5")	RSM	+	-	-

Remarks: SPC stands for SPECTINOMYCIN.

TABLE 8: FUNCTION AND ENZYME ACTIVITY OF PC-BINDING PROTEINS IN *E. coli*

(PBP)	Mr(10^{-3})	(Function)	(Enzyme Activity)
1A	ca. 90	Cell-elongation[1-3]	Transglycosylase-transpeptidase[8,9]
1Bs*	ca. 90	Cell-elongation[1-3]	Transglycosylase-transpeptidase[5-7]
?	66	Formation of rod shape[1]	(Transglycosylase ?-) Transpeptidase[10]
3	60	Formation of septa[1]	Transglycosylase-transpeptidase[11,12]
4	49	Auxiliary[15]	DD-Peptide hydrolase(DD-carboxy-peptidase, DD-endopeptidase)[15]
5	42	Auxiliary[15]	DD-carboxypeptidase[15]
6	40	Auxiliary ?	DD-caboxypeptidase

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TABLE 9: BETA-LACTAM-HYPERSENSITIVITY SHOWN BY PBP-1 Bs-LESS MUTANT STRAINS OF *Escherichia coli*

[Beta-lactam Antibiotic]	[Parent Strain] (JE 1011)	[PBP-1 Bs^- Mutant] (JST 975)	[PBP-1 Bs^+ Revertant] (JST 975-rev 2)	[PBP-1 Bs^+] (CD 4-9752)	[PBP-1 Bs^-] (CD 4-9751)
Penicillin G	30	3	30	30	10
Ampicillin	3	0.3	3	3	1
Cephaloridine	3	0.1	3	3	0.3
Cephalexin	10	1	10	-	-
Cefoxitin	3	0.3	3	3	0.3
Mecillinam	0.3	0.03	0.1	-	-
Nocardicin A	30	3	100	100	1

Remarks: Minimal Inhibitory Concentration is shown by serial dilution.

TABLE 10: MINIMAL INHIBITORY CONCENTRATION OF ANTIBIOTICS AGAINST *Escherichia coli* NIHJ JC-2 AND ITS MUTANT HYPERSENSITIVE TO PC G

(Antibiotic)	(M.I.C., mcg/ml) NIHJ JC-2	Es-114
Penicillin G	100	3.2
Cephalosporin C	200	0.8
6-APA	200	50
7-ACA	50	12.5
Cephamycin C	25	1.6
Clavulanic Acid	200	50
Thienamycin	2	0.125
Aminobenzyl-PC	6.4	0.8
Carboxybenzyl-PC	50	3.2
Cloxacillin	more than 800	3.2
Mecillinam	0.2	0.2
Cephalothin	6.3	0.4
Cephaloridine	50	1.6
Cephalexin	100	6.3
Cephazolin	6.3	1.6

MIC was measured by paper disc-agar plate diffusion assay.